CONTRACT SERIAL NUMBER 69197-01-ST
DSS REQUISITION NUMBER 23440-6-9197
CONTRACTOR: UNIVERSITY OF CALGARY

I OXYGEN AND SULPHUR ISOTOPE FRACTIONATION DURING BACTERIAL OXIDATION OF METAL SULPHIDES

II OXYGEN ISOTOPE EXCHANGE BETWEEN ARSENATE AND WATER

H. Roy Krouse
Department of Physics
The University of Calgary
2500 University Drive N.W.
Calgary, Alberta
Canada T2N 1N4

Report prepared for
Energy, Mines, and Resources Canada
under
Supply and Services Canada
Contract File Number 38ST.23440-6-9197

51pp

MICROMEDIA

1. Oxygen and Sulphur Isotope Fractionation during Bacterial Oxidation of Metal Sulphides

Executive Summary

Bacterial oxidation experiments with pyrite, sphalerite, chalcopyrite, pentlandite, and ore from Hudson Bay Mining and Smelting were carried out in water with different oxygen isotope compositions.

At one week intervals, SO_4^{2-} in the reaction flasks was sampled to determine the extent of oxidation, sulphur and oxygen isotope composition of the SO_4^{2-}, and oxygen isotope composition of the water.

The sulphur isotope composition of the sulphate was essentially identical to that of the reactant sulphide. This is consistent with surface reactions on the mineral grains which should have negligible sulphur isotope selectivity.

In all cases, the oxygen isotope composition of the sulphate correlated strongly with that of the water according to the following linear equations.

$$\delta^{18}O(SO_4^{2-}) = m\ \delta^{18}O(H_2O) + b$$

Mineral	m	b	r^2
Pyrite (Org. 1)	0.66 ± 0.02	3.6 ± 2.6	0.973
Pyrite (Org. 2)	0.71 ± 0.02	3.7 ± 3.2	0.971
Pentlandite	0.70 ± 0.01	7.0 ± 1.4	0.993
Sphalerite	0.57 ± 0.02	7.7 ± 3.2	0.949
Chalcopyrite	0.37 ± 0.03	4.5 ± 5.0	0.803

Therefore oxygen in the water molecule is an active participant in the oxidation reaction through direct incorporation and/or oxygen exchange with reaction intermediates.

I. Fractionnement isotopique de l'oxygène et du soufre pendant l'oxydation bactérienne de sulfures métalliques

Summaire

Des expériences portant sur l'oxydation bactérienne de la pyrite, la sphalérite, la chalcopyrite, la pentlandite et divers minerais provenant de la compagnie "Hudson Bay Mining and Smelting" fûrent tentées en présence d'eaux à différentes teneurs isotopiques d'oxygène.

A toutes les semaines, les sulfates présents dans les fioles à reáctions fûrent échantillonnés de façon à déterminer, d'une part, le progrès de l'oxydation en cours, et d'autre part, la composition isotopique du soufre et de l'oxygène dans les sulfates, et la composition isotopique de l'oxygène dans l'eau.

Lors de ces expériences, la composition isotopique du soufre dans les sulfates demeura la même que celle des sulfures réactants. Ce résultat est compatible avec des réactions superficielles sur les grains de minéraux, puisque ce genre de réactions n'est accompagné que d'un fractionnement isotopique négligible.

Dans tous les cas, la composition isotopique de l'oxygène dans les sulfates a pu être corrélée de façon fort significative avec celle de l'eau suivant les équations linéaires suivantes:

$$\delta^{18}O(SO_4^{2-}) = m\ \delta^{18}O(H_2O) + b$$

Mineral	m	b	r^2
Pyrite (Org. 1)	0.66 ± 0.02	3.6 ± 2.6	0.973
Pyrite (Org. 2)	0.71 ± 0.02	3.7 ± 3.2	0.971
Pentlandite	0.70 ± 0.01	7.0 ± 1.4	0.993
Sphalérite	0.57 ± 0.02	7.7 ± 3.2	0.949
Chalcopyrite	0.37 ± 0.03	4.5 ± 5.0	0.803

En conséquence, il semble que l'oxygène dans la molécule d'eau agit comme un agent des plus actifs lors de la réaction d'oxydation, soit en si'incorporant directement à la réaction, ou bien indirectement par le biais de réactions intermédiares d'échange de l'oxygène.

II. Oxygen Isotope Exchange between Arsenate and Water

A feasibility study was carried out to ascertain if the oxygen isotope composition of arsenate could be used to elucidate mechanisms of bacterial oxidation of arsenopyrite. A technique was successfully developed for determining the oxygen isotope composition of arsenate. To determine oxygen exchange rates between arsenate and water, $Na_2HAsO_4 \cdot 7H_2O$ was dissolved in waters of different oxygen isotope composition. The arsenate was precipitated with Ag^+. It was found that the exchange was very rapid and was even significant during the time required for dissolution and immediate precipitation. Therefore, the oxygen isotope composition of arsenate cannot be used to study bacterial oxidation of arsenic in arsenopyrite or other arsenic containing minerals.

II. Echange isotopique de l'oxygène entre l'eau et l'arséniate

Summaire

Une étude fût entreprise pour déterminer si la composition isotopique de l'arséniate pourraît être utilisée pour élucider les méchanismes d'oxydation de l'arsenopyrite. Une technique fut dévelopée avec succès pour déterminer la composition isotopique de l'arséniate. $Na_2HAsO_4 \cdot 7H_2O$ fût dissout dans des eaux à différentes teneurs isotopiques d'oxygène de façon à déterminer les taux d'échange de l'oxygène entre l'arséniate et l'eau. L'arseniate fût precipité avec de l'Ag^+. Malheureusement, il fût observé que l'échange était trop rapide, même lors d'une dissolution et precipitation subséquente immediate.

En consequence, la composition isotopique de l'oxygene ne peut etre utilisee dans l'etude de l'oxydation bacterienne de l'arsenic dans l'arsenopyrite, ou tout autre mineraux contenant de l'arsenic.

INTRODUCTION

A better understanding of the mechanisms of bacterial oxidation of sulphide is important for many reasons. On one hand, it is desirable to optimize the process for bacterial leaching recovery of base and precious metals. On the other hand, from the environmental viewpoint, one would like to minimize acid mine drainage from mill tailings.

Stable isotopes provide a convenient tracer for studying biological mechanisms. It has been found that sulphur isotope fractionation during oxidation of metal sulphides or elemental sulphur tends to be minimal. This is consistent with surface reactions proceeding "layer by layer" thereby not providing a sufficiently large pool of dissolved sulphur species for optimal isotopic selectivity.

In contrast, the oxygen isotope composition of sulphate produced during the oxidation is highly variable. This arises because the oxidation is in reality an "oxidation-hydrolysis", i.e. oxygen can be derived from two sources, O_2 and H_2O molecules (cf. Lloyd, 1968; Taylor et al, 1984; van Everdingen and Krouse, 1985). There has been considerable controversy as to whether the water molecule per se performs an oxidizing role or whether it only influences the oxygen isotope composition of the product sulphate through exchange reactions with intermediates. This question has not been satisfactorily answered in the scattered reports in the literature for a number of reasons. Many data are from natural systems where many factors are uncertain. The isotopic composition of water is usually, but not always known. The isotope composition of dissolved O_2 is assumed to be the same as that of atmospheric O_2 which in many cases is incorrect in view of the variety of redox reactions which occur. There may be sources of sulphate in

natural systems additional to that produced by sulphide oxidation. Laboratory studies have been admirable but insufficient.

In order to provide a better data base to assess the bacterial oxidation mechanisms, the following features were incorporated in the current experiments.

(1) Bacterial oxidations were carried out in waters of various oxygen isotope compositions in order to ascertain how well the oxygen isotope composition of the product sulphate correlated to that of the water.

(2) The experiments were maintained for up to 5 weeks with weekly sampling.

(3) In addition to sulphate, the isotope composition of the water was monitored since kinetic isotope effects accompany evaporation.

(4) Whereas previous reports were confined to pyrite, other metal sulphides (sphalerite, pentlandite, chalcopyrite, and mixed sulphide ore) were examined to see if the oxygen isotope composition of the sulphate was influenced by the nature of the reactant sulphide.

MATERIALS AND METHODS

Substrates:

The following substrates were used for these experiments: (1) pyrite #1; Fe 44.15%, S 49.83%, (2) pyrite #2; FE 44.36%, S 50.40%, (3) sphalerite #1; Zn 67.00%, S 32.57, $\delta^{34}S$ -3.1. (4) sphalerite #2; Zn 67.17%, S 32.76%, (5) pentlandite; Fe 37.68%, Ni 14.76%, S 31.22%, (6) chalcopyrite; Fe 30.36%, Cu 35.35%, S 34.64%, $\delta^{34}S$ +0.3 (7) HBMS ore; Fe 26.85%, Zn 0.87%, Ni 0.03%, Cu 0.29%, S 28.07%. The Hudson's Bay Mining and Smelting, Flin Flon, Manitoba (HBMS) ore was pretreated with 2.0N HCl in order to remove calcite.

Procedures:

The Thiobacillus ferrooxidans isolate used in this study (EL-5) was isolated from a mine water sample (McCready et al., 1986). The inocula were maintained on the HBMS ore (6 g ore + 100 ml autotrophic medium) in 250 ml Erlenmeyer flasks at 30° on a rotary shaker (150 RPM). The autotrophic medium used during this study was a sulphate free medium of the following composition: K_2HPO_4 0.1 mM, $MgCl_2$ 0.5mM, NH_4Cl 1.0mM, adjusted to pH 2.5.

The experiments were carried out in 2.8 liter Fernbach flasks and contained: 10 g of sulphide mineral (or 60 g of HBMS ore), 900 mL of medium, and 100 mL of inoculum. Varying amount of $H_2{}^{18}O$ enriched water were also added to some of the samples. The flasks were incubated at 30°C on a rotary

shaker (150 RPM). At various time intervals samples of the supernatant (80 mL unless otherwise specified) were withdrawn and filtered twice through Whatman #42 filter paper: Ten mL of the filtrated was saved for metal analysis, and 25-50 mL of the filtrate for sulphate determination. The metal ion concentrations were analyzed by atomic absorption spectrophotometry using an Instrumentation Laboratories IL 551 instrument. Four mL of concentrated HCl was added to the aliquot taken for sulphate analysis, and the solution was heated to a boil. Then 10 mL of 10% w/v $BaCl_2$ was added to the acidified filtrated and the precipitated barium sulphate was allowed to settle overnight. The barium sulphate was filtered and collected on a 0.45 u Millipore filter, washed with 100 mL of 1 N HCl, 500 mL of deionized water and dried for 24 h at 60°C. The dried barium sulphate was weighed and transferred to small vials for subsequent $\delta^{18}O$ and $\delta^{34}S$ determinations.

Experimental Design:

Experiment No. 1:

Four flasks, each containing 900 mL of medium plus 100 mL of inoculum contained the following substrates: (1) 10 g sphalerite (sample #1) (2) 10 g chalcopyrite (sample #1) (3) 10 g pyrite and (4) 60 g HBMS ore. The flasks were sampled at five time intervals; 6, 14, 21, 28 and 35 days respectively. Sulfate samples 1 to 22 were obtained from this experiment.

Experiment No. 2:

Six flasks, each containing 60 g of HBMS ore in one liter of medium plus inoculum were amended with the following amounts of ^{18}O enriched water: 2, 4, 6, 8, 10 and 15 mL. The flasks were samples at five time intervals: 7, 14, 21, 27 and 35 days respectively. Sulfate samples 1A to 31A were obtained from this experiment.

Experiment No. 3:

Five flasks, each containing 10 g of sphalerite (sample #1) in one liter of medium plus inoculum were amended with the following amounts of ^{18}O enriched water: 2, 4, 6, 8 and 10 mL. The flasks were sampled at four time intervals: 7, 14, 23 and 29 days respectively. Sulfate samples 1B to 21B were obtained from this experiment.

Experiment No. 4:

Three flasks, each containing 10 g of pentlandite in one liter of medium plus inoculum were amended with the following amounts of ^{18}O enriched water: 0, 2 and 10 mL. The flasks were sampled at five time intervals: 7, 15, 21, 27 and 35 days respectively. Sulfate samples 1C to 17C were obtained from this experiment.

Experiment No. 5:

Five flasks, each containing 10 g of pentlandite in one liter of medium plus inoculum were amended with the following amounts of ^{18}O enriched water: 4, 6, 8, 15 and 20 mL. The

flasks were sampled at five time intervals: 7, 15, 21, 28 and 35 days respectively. Sulfate samples 1D to 26D were obtained from this experiment.

Experiment No. 6:

Six flasks, each containing 10 g of pyrite (sample #2) in one liter of medium plus inoculum were amended with the following amounts of ^{18}O enriched water: 0, 2, 4, 6, 8 and 10 mL. The flasks were samples at five time intervals: 7, 14, 21, 28 and 34 days respectively. Sulfate samples 1E to 31E were obtained from this experiment.

Experiment No. 7:

Six flasks, each containing 5 g of pyrite (sample #2) plus 5 g of sphalerite (sample #2) in one liter of medium plus inoculum were amended with the following amounts of ^{18}O enriched water: 0, 2, 4, 6, 8 and 10 mL. The flasks were sampled at five time intervals: 7, 14, 21, 28 and 34 days respectively. Sulfate samples 1F to 30F were obtained from this experiment.

N.B. The ^{18}O labelled water used in experiments 1 to 7 was found to be unlabelled.

Experiment No. 8:

Nine flasks, each containing 10 g of pyrite (sample #2) in one liter of medium plus inoculum were prepared in the following way: (1) 900 mL Calgary water (2) 600 mL Calgary water + 300 mL Ottawa water (3) 300 mL Calgary water + 600 mL

Calgary water (4) 900 mL Ottawa water (5) 900 mL Ottawa water + 0.2 mL $H_2{}^{18}O$ (10% enriched) (6) 900 mL Ottawa water + 0.4 mL $H_2{}^{18}O$ (7) 900 mL Ottawa water + 0.6 mL $H_2{}^{18}O$ (8) 900 mL Ottawa water + 0.8 mL $H_2{}^{18}O$ (9) 900 mL Ottawa water + 1.0 Ml $H_2{}^{18}O$. The flasks were sampled at five time intervals: 6, 13, 20, 27 and 34 days respectively. Sulfate samples 1G to 46G were obtained from this experiment.

Experiment No. 9:

Nine flasks, each containing 10 g of sphalerite (sample #2) in one liter of medium plus inoculum were prepared in the following way: (1) 900 mL Calgary water (2) 600 mL Calgary water + 300 mL Ottawa water (3) 300 mL Calgary water + 600 mL Ottawa water (4) 900 mL Ottawa water (5) 900 mL Ottawa water + 0.2 mL $H_2{}^{18}O$ (6) 900 mL Ottawa water + 0.4 mL $H_2{}^{18}O$ (7) 900 mL Ottawa water + 0.6 mL $H_2{}^{18}O$ (8) 900 mL Ottawa water + 0.8 ml $H_2{}^{18}O$ (9) 900 mL Ottawa water + 1.0 mL $H_2{}^{18}O$. The flasks were sampled at five time intervals: 6, 13, 20, 27 and 34 days respectively. Sulfate samples 1H to 45H were obtained from this experiment.

Experiment No. 10:

Nine flasks, each containing 10 g of pentlandite in one liter of medium plus inoculum were prepared in the following way: (1) 900 mL Calgary water (2) 600 mL Calgary water + 300 mL Ottawa water (3) 300 mL Calgary water + 600 mL Ottawa water (4) 900 mL Ottawa water (5) 900 mL Ottawa water + 0.2 mL $H_2{}^{18}O$ (6) 900 mL Ottawa water + 0.4 mL $H_2{}^{18}O$ (7) 900 mL Ottawa water + 0.6 mL $H_2{}^{18}O$ (8) 900 mL Ottawa water + 0.8 mL $H_2{}^{18}O$ (9) 900 mL Ottawa water + 1.0 mL $H_2{}^{18}O$. The

flasks were sampled at four time intervals: 6, 13, 20, and 27 days respectively. Sulphate samples 1I to 37I were obtained from this experiment.

Experiment No. 11

Nine flasks, each containing 10 g of chalcopyrite in one liter of medium plus inoculum were prepared in the following way: (1) 900 mL Calgary water (2) 600 mL Calgary water + 300 mL Ottawa water (3) 300 mL Calgary water + 600 mL Ottawa water (4) 900 mL Ottawa water (5) 900 mL Ottawa water + 0.2 mL $H_2{}^{18}O$ (6) 900 mL Ottawa water + 0.4 mL $H_2{}^{18}O$ (7) 900 mL Ottawa water + 0.6 mL $H_2{}^{18}O$ (8) 900 mL Ottawa water + 0.8 mL $H_2{}^{18}O$ (9) 900 mL Ottawa water + 1.0 mL $H_2{}^{18}O$. The flasks were sampled at five time intervals: 6, 13, 27, 41, and 55 days respectively. Sulphate samples 1J to 46J were obtained from this experiment.

Experiment No. 12

Nine flasks, each containing 5 g of pentlandite plus 5 g of chalcopyrite in one liter of medium plus inoculum were prepared in the following way:
(1) 900 mL Calgary water (2) 600 mL Calgary water + 300 mL Ottawa water (3) 300 mL Calgary water + 600 mL Ottawa water (4) 900 mL Ottawa water (5) 900 mL Ottawa water + 0.2 mL $H_2{}^{18}O$ (6) 900 mL Ottawa water + 0.4 mL $H_2{}^{18}O$ (7) 900 mL Ottawa water + 0.6 mL $H_2{}^{18}O$ (8) 900 mL Ottawa water + 0.8 mL $H_2{}^{18}O$ (9) 900 mL Ottawa water + 1.0 mL $H_2{}^{18}O$. The flasks were sampled at six time intervals: 7, 14, 21, 28, 35, and 42 days respectively. Sulphate samples 1K to 55K were obtained from this experiment.

Experiment No. 13

This experiment on pyrite oxidation was essentially identical to No. 8 except that a different strain of T. ferrooxidans was used (SM-4). The flasks were sampled at five time intervals: 7, 14, 21, 28, and 35 days. Sulphate samples 1L to 46L were obtained from these experiments.

Mass Spectrometry

$BaSO_4$ was converted to SO_2 for sulphur isotope analyses using the high temperature decomposition procedure of Ueda and Krouse (1987).

For oxygen isotope analyses, $BaSO_4$ was reduced by graphite in platinum boats through which currents of the order of 20A were passed. both CO_2 and CO were produced in this high temperature reaction. The CO was converted to CO_2 using a high voltage discharge unit and a liquid N_2 bath. The apparatus is a variation of that described by Sakasi and Krouse (1971).

Sulphur and oxygen isotope analyses were obtained with mass spectrometers that have been constructed around Micromass 602 and Micromass 903 basic components.

RESULTS

The results are presented in Tables 1 to 13 and Figures 1 to 6. Linear fits to the data of Experiments No. 8 to No. 13 are given in Table 14.

TABLE 1 Experiment #1

OXIDATION OF VARIOUS SULPHIDE MINERALS AND ORE

(Ottawa River Water, $\delta^{18}O = -11.1^{o}/oo$)

Sample	Time (Days)	SO_4^{2-} (mmol/mg)	$\delta^{34}S$	$\delta^{18}O$, SO_4^{2-} meas.	cor.
Inoculum 1	0	2.31	-1.5	+9.9	
Chalcopyrite					
3	6	1.87	-0.9	+9.3	+6.7
7	14	2.99	-0.6	+7.0	+1.6
11	21	3.87	-0.9	+7.2	+3.9
15	28	4.05	-0.5	+4.9	+0.4
19	35	5.36	+0.1	+2.1	-3.1
		AVERAGE:	-0.6 ± 0.4		+1.9 ± 3.7
Pyrite			+1.9		
4	6	3.62	+0.5	+4.2	-5.9
8	14	8.55	+1.0	-0.3	-4.1
12	21	13.54	+2.1	-1.1	-3.4
16	28	21.27	+1.8	-2.7	-4.2
20	35	31.44	+2.0	+4.2	3.7
		AVERAGE:	+0.5 ± 0.7		-2.8 ± 3.7
Sphalerite					
5	6	8.30	-2.1	+6.1	+5.1
9	14	15.78	-2.6	+4.7	+3.9
13	21	19.59	-2.4	+1.1	0.0
17	28	22.70	-2.8	+2.7	+1.9
21	35	25.89	-3.0	+1.9	+1.1
		AVERAGE:	-2.6 ± 0.4		+2.4 ± 2.1
HBMS Ore			+0.3		
6	6	10.04	-0.2	+1.2	-1.4
10	14	27.07	+0.5	+4.7	0.4
14	21	48.84	+0.3	-1.5	-2.1
18	28	84.89	+0.1	-	-
22	35	135.42	+0.5	-2.5	-2.7
		AVERAGE:	0.2 ± 0.3		-1.5 ± 1.3

TABLE 2 Experiment #2

OXIDATION OF HUDSON BAY MINING AND SMELTING ORE

(Ottawa River Water, $\delta^{18}O = -11.1°/_{oo}$)

Sample	Time(Days)	SO_4^{2-}(mmol/mg)	$\delta^{34}S$	$\delta^{18}O$ SO_4^{2-}	$\delta^{18}O$ H_2O
Inoculum 1A	0	1.04	-0.1	+0.7	-10.5
2A (2 ml)	7	13.35	+0.2	-3.3	-11.1
8A	14	69.99	+0.2	-4.5	
14A	21	122.26	+0.6	-3.4	
20A	27	142.84	+0.5		
26A	35	149.26	+0.3	-3.2	
3A (4 ml)	7	14.03	-0.1	-1.8	-11.1
9A	14	67.74	+0.3	-3.8	
15A	21	124.13	+0.5	-2.2	
21A	27	144.55	+0.3	-4.3	
27A	35	156.22	+0.1	-2.2	
4A (6 ml)	7	12.41	+0.1	-3.6	-10.5
10A	14	66.24	+0.2	-3.0	
16A	21	114.90	+0.2	-3.5	
22A	27	136.35	+0.2		
28A	35	145.33	-0.1		
5A (8 ml)	7	12.23	+0.4	-0.3	-11.2
11A	14	60.69	+0.3	-4.4	
17A	21	112.40	+0.3	-4.2	
23A	27	135.23	+0.1	-1.3	
29A	35	147.17	+0.2		
6A (10 ml)	7	13.91	0.0	-4.4	-9.9
12A	14	64.93	+0.3	-4.4	
18A	21	115.08	+0.3	-2.4	
24A	27	137.26	+0.3	-1.2	
30A	35	149.26	+0.4		
7A (15 ml)	7	12.10	+0.1	-3.7	
13A	14	58.88	+0.3	-1.9	
19A	21	112.90	+0.5	-3.1	
25A	27	133.67	+0.2	-3.6	
31A	35	144.27	+0.2	-2.9	
OVERALL AVERAGE:			+0.3 ± 0.2	-3.1 ± 1.1	

TABLE 3 Experiment #3

OXIDATION OF SPHALERITE

(Ottawa River Water, $\delta^{18}O = -11.1°/_{oo}$)

Sample	Time(Days)	SO_4^{2-}(mmol/mg)	$\delta^{34}S$	$\delta^{18}O$ SO_4^{2-}	$\delta^{18}O$ H_2O
Inoculum 1B	0	9.54	+0.2	+0.3	-10.6
2B (2 ml)	7	30.36	-1.6	-5.2	-10.4
7B	14	33.50	-2.5	-5.6	
12B	23	44.72	-2.7	-4.0	
17B	29	47.69	-2.7	-4.7	
3B (4 ml)	7	29.75	-2.1	-3.0	-10.5
8B	14	32.59	-2.2	-5.6	
13B	23	44.13	-2.7	-6.5	
18B	29	46.13	-2.8	-5.5	
4B (6 ml)	7	31.19	-2.3	-2.5	-10.6
9B	14	29.69	-2.2	-1.4	
14B	23	44.91	-2.4	-3.6	
19B	29	45.69	-2.8	-5.2	
5B (8 ml)	7	26.26	-2.0	-3.0	-10.4
10B	14	28.54	-1.6	-4.3	
15B	23	37.71	-2.4	-4.0	
20B	29	40.48	-2.5	-2.8	
6B (10 ml)	7	27.07	-2.1	-3.3	
11B	14	25.79	-2.2	-4.5	
16B	23	41.73	-2.5	-4.6	
21B	29	43.29	-2.7	-4.3	
OVERALL AVERAGE:			-2.4 ± 0.4	-4.2 ± 1.3	

TABLE 4 Experiment #4

OXIDATION OF PENTLANDITE

(Ottawa River Water, $\delta^{18}O = -11.1°/oo$)

Sample	Time(Days)	SO_4^{2-}(mmol/mg)	$\delta^{34}S$	$\delta^{18}O$ SO_4^{2-}	$\delta^{18}O$ H_2O
Inoculum 1C	0	2.31	+1.6	-2.5	-10.8
2C (0 ml)	7	52.58	+2.7	-6.2	-10.4
6C	14	88.70	+3.0	-6.5	-10.9
9C	21	96.84	+3.1	-4.8	
12C	27	104.42	+3.1	-6.4	
15C	35	110.31	+3.2	-4.5	
3C (2 ml)	7	50.62	+2.7	-4.1	-10.6
7C	14	90.26	+2.9	-6.5	
10C	21	97.96	+2.8	-5.7	
13C	27	104.60	+3.2	-5.7	
16C	35	111.22	+2.9	-5.0	
4C (4 ml)	7	50.43	+2.6	-6.4	
5C (10 ml)	7	49.74	+2.8	-6.3	
8C	14	86.89	+3.1	-4.4	
11C	21	96.59	+3.0	-4.9	
14C	27	102.42	+2.8	-3.1	
17C	35	110.65	+2.9	-5.7	
		OVERALL AVERAGE:	2.9 ± 0.2	-5.4 ± 1.0	

TABLE 5 Experiment #5

OXIDATION OF PENTLANDITE

(Ottawa River Water, $\delta^{18}O = -11.1°/_{oo}$)

Sample	Time(Days)	SO_4^{2-}(mmol/mg)	$\delta^{34}S$	$\delta^{18}O$ SO_4^{2-}	$\delta^{18}O$ H_2O
Inoculum 1D	0	2.28	+1.3	+0.1	-10.3
2D (4 ml)	7	18.12	+2.3	-2.8	-10.5
7D	15	29.50	+2.0	-4.4	
12D	21	38.67	+2.5		
17D	28	49.37	+2.4	-2.3	
22D	35	66.55	+2.6	-2.8	
3D (6 ml)	7	15.66	+1.9	-3.5	-10.3
8D	15	28.35	+2.3	-2.6	
13D	21	34.09	+2.0	-2.8	
18D	28	44.54	+2.3	-4.1	
23D	35	65.53	+2.6	+0.3	
4D (8 ml)	7	18.90	+2.1	-4.3	-10.6
9D	15	32.62	+2.3	-3.2	
14D	21	44.44	+2.5	-3.4	
19D	28	61.19	+2.4	-2.3	
24D	35	83.36	+2.7	-2.6	
5D (15 ml)	7	17.62	+1.7	-3.6	-10.4
10D	15	28.07	+2.2	-1.2	
15D	21	34.46	+2.1	-3.5	
20D	28	43.48	+2.3	-3.5	
25D	35	60.41	+2.6	-3.5	
6D (20 ml)	7	18.34	+1.7	-3.4	
11D	15	28.38	+2.0	-0.4	
16D	21	34.24	+2.1	-4.5	
21D	28	42.23	+2.2	-2.5	
26D	35	92.60	+2.6	-3.7	
OVERALL AVERAGE:			2.3 ± 0.3	-2.9 ± 1.2	

TABLE 6 Experiment #6

OXIDATION OF PYRITE #2

(Ottawa River Water, $\delta^{18}O = -11.1^{o}/oo$)

Sample	Time (Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$, SO_4^{2-}		H_2O
			meas.	cor.	
Inoculum 1E	0	12.23	-0.6		-9.8
2E (0 ml)	7	49.31	-1.4	-1.7	-9.6
8E	14	71.92	-1.9	-2.2	
14E	21	89.48	-3.0	-3.4	
20E	28	104.29	-3.0	-3.3	
26E	34	117.61	-2.2	-2.4	
3E (2 ml)	7	54.70	-2.0	-2.4	-9.8
9E	14	77.35	-3.3	-3.8	
15E	21	92.07	-4.0	-4.5	
21E	28	106.01	-1.5	-1.6	
27E	34	119.54	-2.4	-2.6	
4E (4 ml)	7	51.80	+0.2	0.4	-10.0
10E	14	80.71	-3.0	-3.4	
16E	21	95.62	-4.0	-4.5	
22E	28	107.60	-0.7	-0.7	
29E	34	120.51	-3.6	-3.9	
5E (6 ml)	7	47.09	-0.2	-0.1	-9.7
11E	14	79.68	-2.0	-2.3	
17E	21	95.81	-3.8	-4.3	
23E	28	105.26	-2.9	-3.2	
29E	34	120.95	-3.0	-3.3	
6E (8 ml)	7	52.05	-0.8	-0.9	
12E	14	80.37	-3.6	-4.1	
18E	21	92.53	-	-	
24E	28	104.17	-2.8	-3.1	
30E	34	116.42	-2.3	-2.5	
7E (10ml)	7	50.96	-2.4	-3.0	
13E	14	73.63	-2.8	-3.2	
19E	21	92.35	-3.4	-3.8	
25E	28	105.88	-3.1	-3.4	
31E	34	118.14	-2.8	-3.1	
			AVERAGE:	-2.8 ± 1.3	

TABLE 7 Experiment #7

OXIDATION OF PYRITE PLUS SPHALERITE

Sample	Time (Days)	SO_4^{2-} (nmol/mg)	$\delta^{18}O$ SO_4^{2-}	$\delta^{18}O$ H_2O
1E Inoculum	0	12.23	-0.6	-9.8
1F (0 ml)	7	47.8	-1.1	-9.3
7F	14	63.9	-0.5	
13F	21	74.4	-1.3	
19F	28	87.2	+1.6	
25F	34	93.0	-0.2	
2F (2 ml)	7	41.9	-3.1	-9.3
8F	14	57.6	-1.3	
14F	21	61.1	-1.4	
20F	28	84.8	-0.1	
26F	34	91.5	-1.2	
3F (4 ml)	7	49.1	-2.3	-9.2
9F	14	63.5	-1.3	
15F	21	75.7	-2.0	
21F	28	82.5	-0.1	
27F	34	94.0	-0.4	
4F (6 ml)	7	45.2	-0.8	-9.0
10F	14	58.9	-1.2	
16F	21	72.0	+1.0	
22F	28	82.4	-1.1	
28F	34	91.1	-2.0	
5F (8 ml)	7	46.6	-2.0	-9.6
11F	14	59.2	-0.6	
17F	21	71.1	-1.6	
23F	28	81.3	-1.4	
29F	34	90.3	-1.8	
6F (10 ml)	7	45.0	+1.0	-9.6
12F	14	58.1	+0.8	
18F	21	68.4	-0.1	
24F	28	78.6	-3.6	
30F	34	83.8	-2.1	
		OVERALL AVERAGE:	-1.0 ± 1.2	

TABLE 8 Experiment #8

OXIDATION OF PYRITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	$\delta^{18}O$ SO_4^{2-} cor.	$\delta^{18}O$ H_2O meas.	$\delta^{18}O$ H_2O avg.
1G Inoculum		0	2.73	-1.5		-17.6	
2G		6	0.89	-3.9	-5.0	-16.5	-17.1
11G	900 ml	13	1.70	-4.4	-5.0	-15.5	-16.5
20G	Calgary	20	2.15	-4.9	-5.4	-14.9	-16.1
29G	H_2O	27	2.49	-5.6	-6.1	-13.4	-15.6
38G		34	2.52	-6.9	-7.6	-13.8	-15.3
		0				-14.7	
3G		6	0.90	-3.6	-4.5	-14.3	-14.5
12G	600 ml	13	1.66	-2.9	-3.2	-13.3	-14.1
21G	Calgary	20	2.09	-6.1	-6.8	-12.9	-13.8
30G	H_2O	27	2.39	-7.4	-8.2	-12.3	-13.5
39G		34	2.55	-5.7	-6.2	-12.4	-13.3
		0				-11.8	
4G		6	0.91	-4.6	-5.9	-11.2	-11.5
13G	300 ml	13	1.65	-1.9	-2.0	-11.0	-11.3
22G	Calgary	20	2.00	-0.4	-0.2	-10.5	-11.1
31G	H_2O	27	2.31	-4.3	-4.7	-9.6	-10.8
40G		34	2.50	-2.8	-3.0	-9.5	-10.6
		0				-8.9	
5G		6	0.92	-2.1	-2.4	-8.7	-8.8
14G	Ottawa	13	1.64	-1.8	-1.9	-8.4	-8.7
23G	Water	20	2.05	-0.5	-0.3	-7.9	-8.5
32G		27	2.43	-2.8	-3.0	-6.6	-8.1
41G		34	2.47	-1.9	-1.9	-5.9	-7.7
		0				+4.1	
6G	0.2 ml	6	0.92	+0.8	+1.8	+4.4	+4.3
15G	$H_2{}^{18}O$	13	1.60	+6.3	+7.9	+4.9	+4.5
24G		20	2.00	+6.6	+7.9	+4.4	+4.5
33G		27	2.40	+3.8	+4.5	+5.8	+4.7
42G		34	2.42	+4.8	+5.6	+6.8	+5.1

TABLE 8 Experiment #8 CONTINUED

OXIDATION OF PYRITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	cor.	$\delta^{18}O$ H_2O meas.	avg.
		0				+14.3	
7G	0.4 ml	6	0.94	+7.5	+11.2	+14.7	+14.5
16G	$H_2{}^{18}O$	13	1.65	+8.7	+10.7	+14.7	+14.6
25G		20	2.05	+8.7	+10.3	+14.8	+14.6
34G		27	2.36	+13.8	+15.8	+15.6	+14.8
43G		34	2.37	+12.3	+14.0	+15.4	+14.9
		0				+26.5	
8G	0.6 ml	6	0.97	+12.0	+17.3	+26.9	+26.7
17G	$H_2{}^{18}O$	13	1.57	+16.6	+20.4	+26.2	+26.5
26G		20	1.99	+19.1	+22.4	+26.7	+26.6
35G		27	2.27	+19.2	+22.0	+28.2	+26.9
44G		34	1.90	+19.9	+23.5	+28.0	+27.1
		0				+38.5	
9G	0.8 ml	6	0.94	+16.4	+23.7	+38.8	+38.7
18G	$H_2{}^{18}O$	13	1.54	+23.6	+28.9	+38.7	+38.7
27G		20	1.89	+24.7	+29.1	+38.5	+38.6
36G		27	2.23	+27.3	+31.3	+40.5	+39.0
45G		34	2.18	+28.2	+32.4	+39.7	+39.1
		0				+49.0	
10G	1.0 ml	6	0.98	+20.8	+29.5	+49.6	+49.3
19G	$H_2{}^{18}O$	13	1.51	+30.3	+37.3	+49.8	+49.5
28G		20	1.86	+32.7	+38.6	+48.8	+49.3
37G		27	2.24	+34.9	+40.0	+50.1	+49.5
46G		34	2.06	+36.5	+42.3	+49.0	+49.4

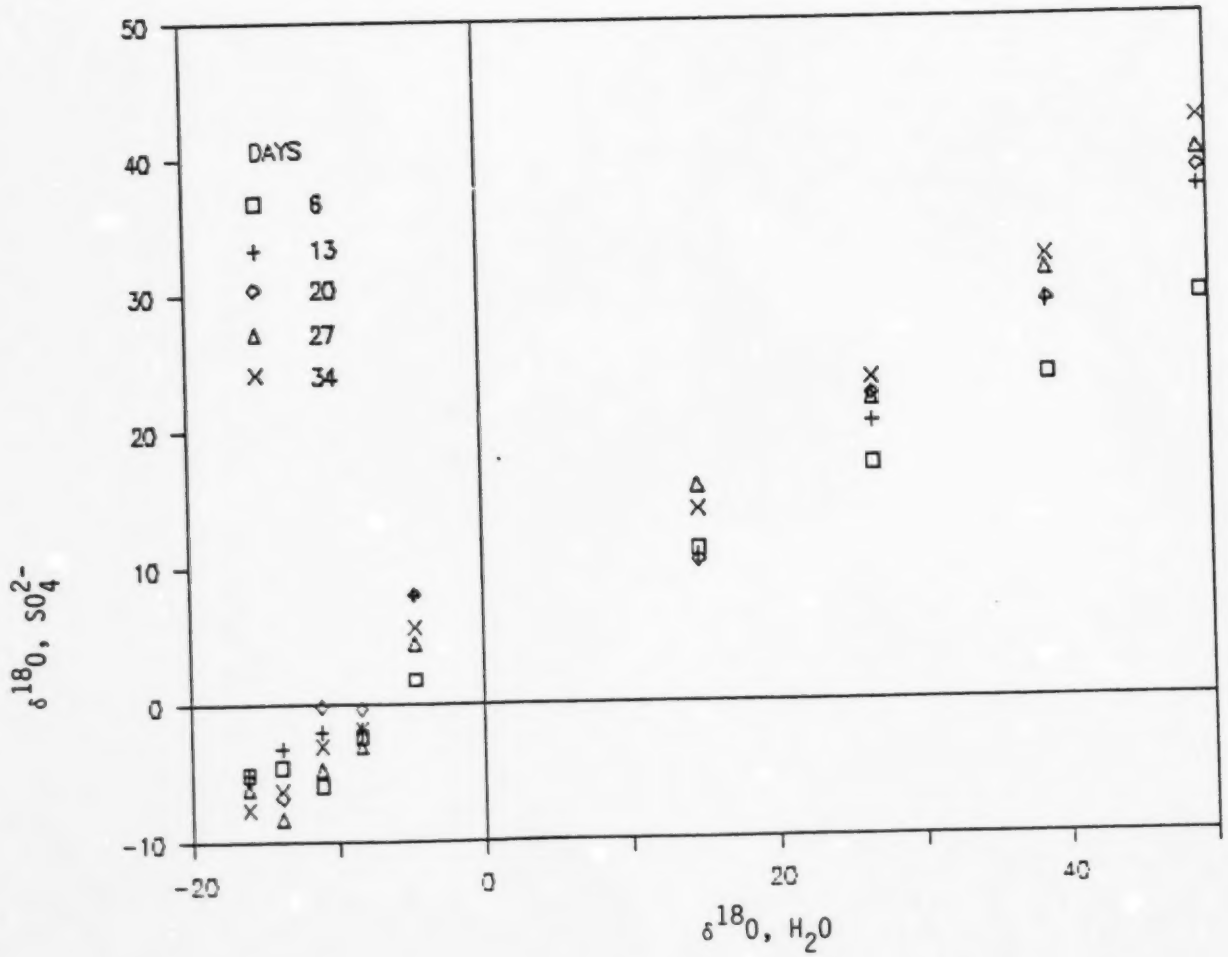

Figure 1 $\delta^{18}O(SO_4^{2-})$ versus $\delta^{18}(H_2O)$

Experiment #8 Oxidation of Pyrite

TABLE 9 Experiment #9

OXIDATION OF SPHALERITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	$\delta^{18}O$ SO_4^{2-} cor.	$\delta^{18}O$ H_2O meas.	$\delta^{18}O$ H_2O avg.
1G Inoculum		0	2.73	-1.5		-17.6	
1H		6	0.87	-1.4	-1.4	-16.6	-17.1
10H	900 ml	13	1.07	-1.9	-2.0	-17.1	-17.1
19H	Calgary	20	1.24	-1.7	-1.8	-16.6	-17.0
28H	H_2O	27	1.32	-2.9	-3.3	-15.4	-16.7
37H		34	1.45	+5.8	+7.5	-15.1	-16.4
		0				-14.8	
2H		6	0.64	-0.5	+0.3	-14.4	-14.6
11H	600 ml	13	0.78	-3.9	-5.2	-12.5	-13.9
20H	Calgary	20	1.21	+0.2	+0.7	-12.7	-13.6
29H	H_2O	27	1.30	-3.6	-4.2	-13.1	-13.5
38H		34	1.44	+0.2	+0.6	-10.7	-13.0
		0				-11.9	
3H		6	0.63	-0.9	-0.4	-11.3	-11.6
12H	300 ml	13	0.80	-2.0	-2.3	-11.7	-11.6
21H	Calgary	20	1.29	+3.3	+4.6	-12.0	-11.7
30H	H_2O	27	1.42	-0.3	0.0	-9.7	-11.3
39H		34	1.49	+3.4	+4.5	-9.2	-11.0
		0				-9.3	
4H		6	0.65	-0.3	+0.6	-8.5	-8.9
13H	Ottawa	13	0.90	0.0	+0.7	-7.7	-8.5
22H	Water	20	1.21	0.0	+0.4	-7.6	-8.3
31H		27	1.29	+0.4	+0.9	-6.8	-8.0
40H		34	1.47	+2.1	+2.9	-5.4	-7.6
		0				+4.1	
5H	0.2 ml	6	0.77	+4.5	+7.8	+4.8	+4.5
14H	$H_2{}^{18}O$	13	1.10	+5.8	+8.2	+5.8	+4.9
23H		20	1.27	+9.3	+12.3	+10.3	+6.3
32H		27	1.42	+7.4	+9.5	+5.9	+6.2
41H		34	1.59	+7.9	+9.9	+7.6	+6.4

TABLE 9 Experiment #9 CONTINUED

OXIDATION OF SPHALERITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	cor.	$\delta^{18}O$ H_2O meas.	avg.
		0				+13.4	
6H	0.4 ml	6	0.61	+4.6	+9.5	+13.8	+13.6
15H	$H_2{}^{18}O$	13	0.77	+6.3	+10.6	+13.9	+13.7
24H		20	1.22	+10.3	+13.7	+15.3	+14.1
33H		27	1.36	+12.3	+15.8	+14.9	+14.3
42H		34	1.48	+12.0	+15.1	+16.1	+14.6
		0				+27.3	
7H	0.6 ml	6	0.84	+12.8	+19.7	+28.0	+27.7
16H	$H_2{}^{18}O$	13	1.13	+17.7	+23.8	+30.7	+28.7
25H		20	1.31	+19.2	+24.7	+28.7	+28.7
34H		27	1.49	+20.7	+25.7	+29.7	+28.9
43H		34	1.66	+20.7	+25.1	+30.5	+29.2
		0				+40.0	
8H	0.8 ml	6	0.78	+16.7	+26.7	+40.0	+40.0
17H	$H_2{}^{18}O$	13	1.10	+22.6	+30.6	+40.5	+40.2
26H		20	1.29	+26.3	+33.8	+40.6	+40.3
35H		27	1.47	+27.6	+34.3	+42.2	+40.7
44H		34	1.64	+30.8	+37.2	+41.9	+40.9
		0				+53.0	
9H	1.0 ml	6	0.87	+22.7	+33.7	+53.2	+53.1
18H	$H_2{}^{18}O$	13	1.07	+30.6	+41.6	+54.1	+53.4
27H		20	1.28	+30.6	+39.4	+53.9	+53.6
36H		27	1.45	+31.6	+39.3	+54.5	+53.7
45H		34	1.64	+34.2	+41.3	+54.4	+53.8

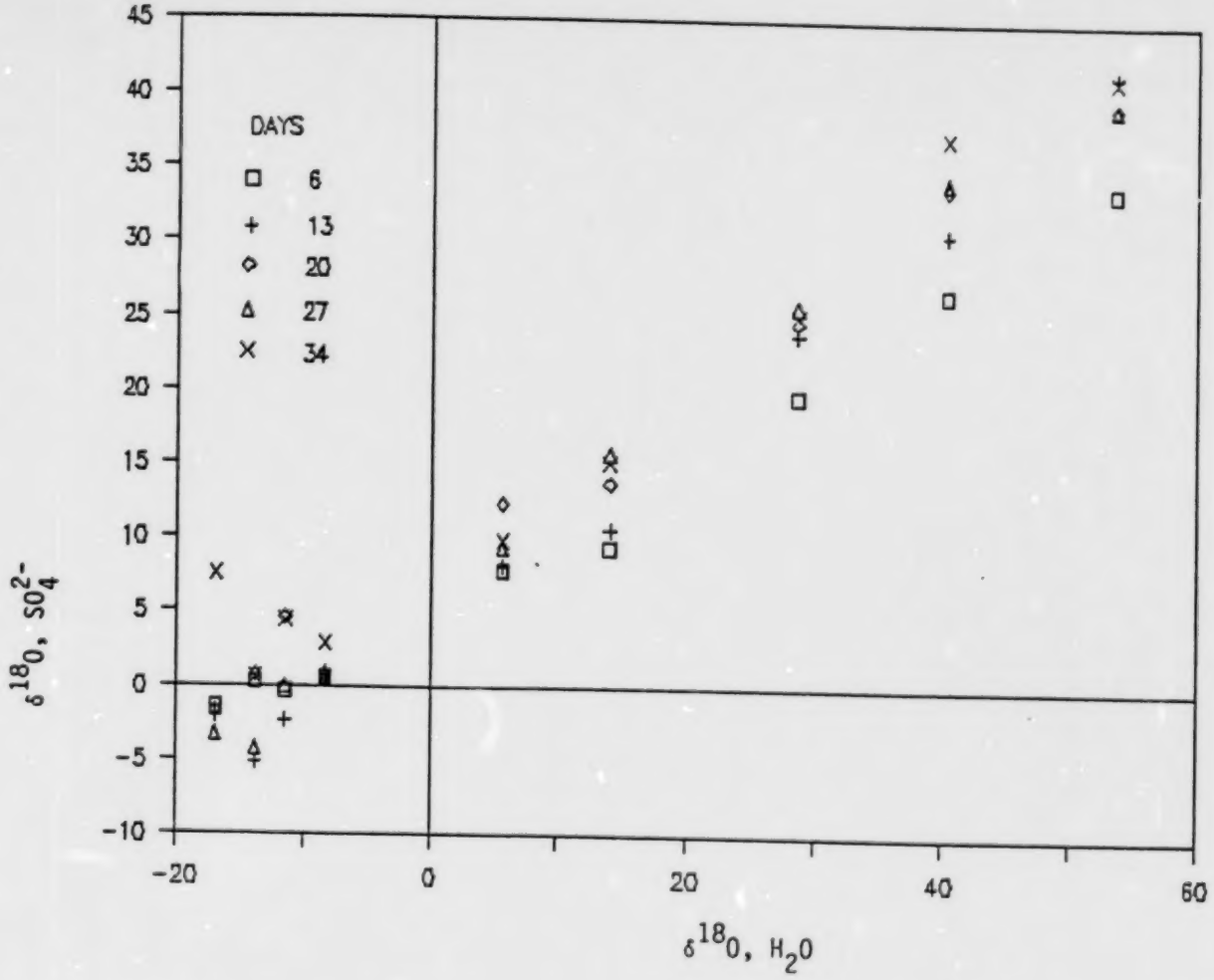

Figure 2 $\delta^{18}O(SO_4^{2-})$ versus $\delta^{18}(H_2O)$

Experiment # 9 Oxidation of Sphalerite

TABLE 10 Experiment #10

OXIDATION OF PENTLANDITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	$\delta^{18}O$ SO_4^{2-} cor.	$\delta^{18}O$ H_2O meas.	$\delta^{18}O$ H_2O avg.
1I Inoculum		0	1.73	+0.3		-16.2	
2I		6	1.31	-2.6	-3.0	-15.9	-16.1
11I	900 ml	13	1.65	-4.6	-5.2	-15.5	-15.9
20I	Calgary	20	1.92	-4.4	-4.9	-13.8	-15.4
29I	H_2O	27	1.93	-4.9	-5.4	-14.1	-15.1
		0				-13.8	
3I		6	1.16	-3.0	-3.6	-14.1	-14.0
12I	600 ml	13	1.65	-2.6	-2.9	-12.8	-13.6
21I	Calgary	20	1.94	-1.0	-1.1	-11.4	-13.0
30I	H_2O	27	1.98	-2.9	-3.2	-12.3	-12.9
		0				-11.7	
4I		6	1.12	+1.0	-0.3	-11.2	-11.5
13I	300 ml	13	1.59	+0.9	+1.0	-10.3	-11.1
22I	Calgary	20	1.86	-1.0	-1.1	-9.7	-10.7
31I	H_2O	27		+1.0	+1.1	-8.6	-10.3
		0				-8.8	
5I		6	1.31	+0.7	+0.8	-8.7	-8.8
14I	Ottawa	13	1.71	+1.1	+1.2	-8.8	-8.8
23I	Water	20	1.93	+2.2	+2.4	-6.5	-8.2
32I		27	1.93	-0.3	-0.4	-6.4	-7.8
		0				+2.8	
6I	0.2 ml	6	1.27	+9.0	+10.4	+3.0	+2.9
15I	$H_2{}^{18}O$	13	1.66	+8.3	+9.2	+3.2	+3.0
24I		20	1.78	+8.1	+8.9	+4.7	+3.4
33I		27	1.94	+9.3	+10.1		

TABLE 10 Experiment #10 CONTINUED

OXIDATION OF PENTLANDITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	cor.	$\delta^{18}O$ H_2O meas.	avg.
		0				+14.7	
7I	0.4 ml	6	1.29	+14.8	+17.0	+15.2	+15.0
16I	$H_2{}^{18}O$	13	1.70	+16.4	+18.2	+15.3	+15.1
25I		20	1.97	+16.1	+17.6	+16.3	+15.4
34I		27	1.93	+15.6	+17.1	+16.1	+15.5
		0				+25.6	
8I	0.6 ml	6	1.25	+20.8	+24.1	+26.7	+26.8
17I	$H_2{}^{18}O$	13	1.66	+23.3	+26.0	+26.2	+26.6
26I		20	1.93	+23.5	+25.8	+27.4	+26.8
35I		27	2.00	+22.1	+24.2	+26.9	+26.8
		0				+36.5	
9I	0.8 ml	6	1.28	+27.8	+32.1	+36.3	+36.4
18I	$H_2{}^{18}O$	13	1.65	+28.6	+31.9	+36.8	+36.5
27I		20	1.95	+32.5	+35.6	+37.2	+36.7
36I		27	2.12	+31.6	+34.4	+37.1	+36.8
		0				+49.0	
10I	1.0 ml	6	1.24	+35.9	+41.7	+50.3	+49.7
19I	$H_2{}^{18}O$	13	1.68	+37.7	+42.0	+51.0	+50.1
28I		20	1.93	+35.4	+38.9	+51.7	+50.5
37I		27	2.01	+42.3	+46.2	+50.6	+50.5

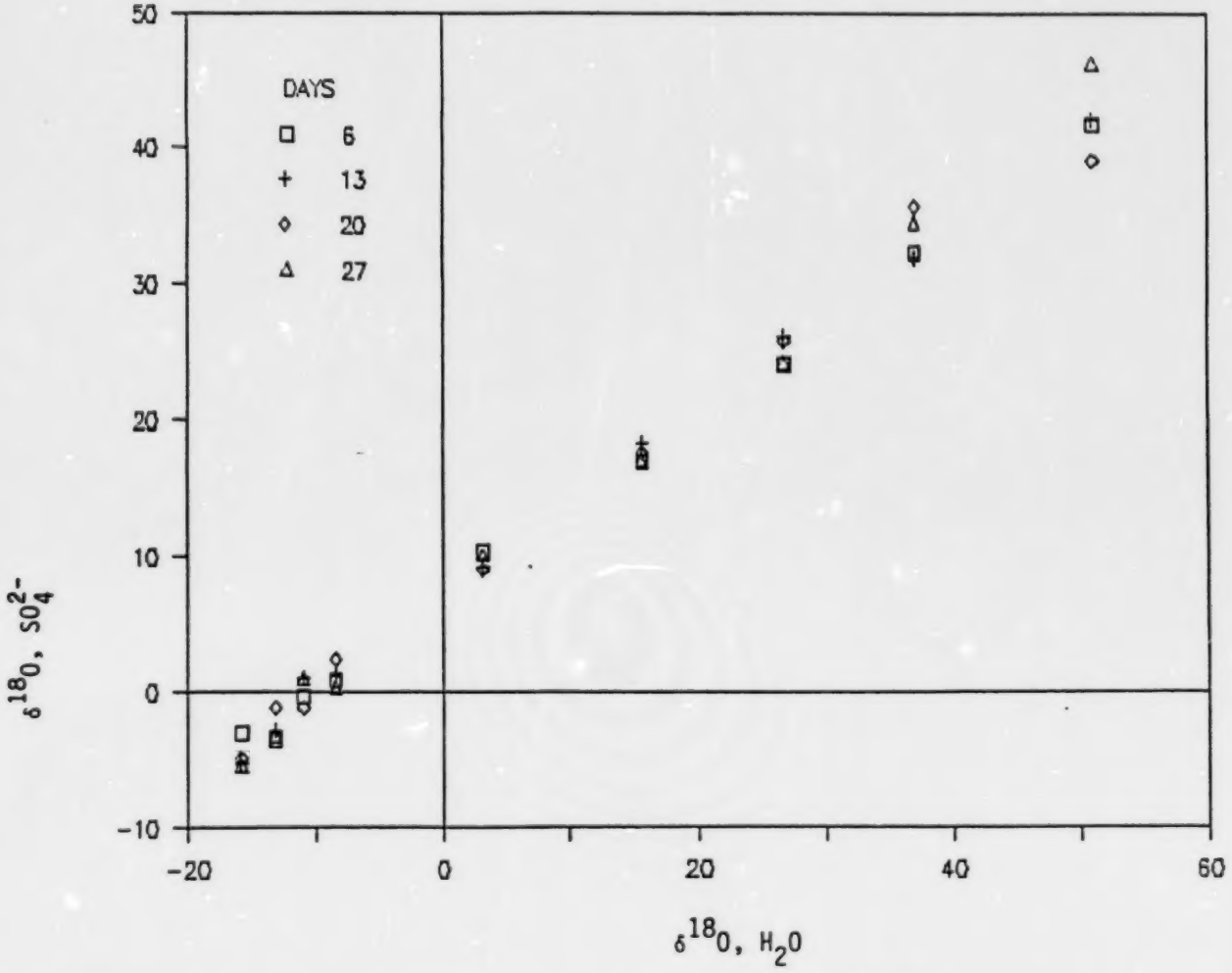

Figure 3 $\delta^{18}O(SO_4^{2-})$ versus $\delta^{18}(H_2O)$

Experiment # 10 Oxidation of Pentlandite

TABLE 11 Experiment #11

OXIDATION OF CHALCOPYRITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	cor.	H_2O meas.	avg.
1J Inoculum		0	2.50	+1.9		-17.5	
		0				-17.5	
2J		6	0.36	+0.8	-1.6	-16.1	-16.8
11J	900 ml	13	0.40	+0.7	-1.3	-14.2	-15.9
20J	Calgary	27	0.46	-1.8	-6.3	-14.5	-15.6
29J	H_2O	41	0.53	-2.3	-6.1	-12.6	-15.0
38J		55	0.60	-1.9	-4.6	-10.7	-14.3
		0				-12.8	
3J		6	0.37	+0.2	-3.4	-14.2	-13.5
12J	600 ml	13	0.39	+4.5	+9.0	-12.2	-13.1
21J	Calgary	27	0.45	+1.8	+1.7	-11.3	-12.6
30J	H_2O	41	0.52	+1.0	+0.2	-11.1	-12.3
39J		55	0.60	+2.4	+2.8	-10.7	-12.1
		0				-12.1	
4J		6	0.36	+1.6	+8.9	-10.6	-11.4
13J	300 ml	13	0.39	+0.8	-1.0	-9.6	-10.8
22J	Calgary	27	0.46	+3.6	+5.7	-9.8	-10.5
31J	H_2O	41	0.51	-0.8	-3.4	-9.2	-10.3
40J		55	0.59	-0.6	-2.4	-7.7	-9.8
		0				-9.4	
5J		6	0.37	+1.6	+1.0	-8.5	-9.0
14J	Ottawa	13	0.39	+0.2	-3.0	-7.8	-8.6
23J	Water	27	0.46	+0.7	-0.7	-7.6	-8.3
32J		41	0.52	+2.6	+3.2	-5.5	-7.8
41J		55	0.61	+7.0	+10.0	-4.2	-7.2
		0				+5.6	
6J		6	0.35	+1.3	-0.2	+7.6	+6.6
15J	0.2 ml	13	0.38	+1.3	+0.1	+7.4	+6.9
24J		27	0.45	+6.8	+13.0	+8.1	+7.2
33J	$H_2{}^{18}O$	41	0.51	+8.8	+15.3	+8.8	+7.5
42J		55	0.62	+3.4	+4.4	+10.0	+7.9

TABLE 11 Experiment #11 CONTINUED

OXIDATION OF CHALCOPYRITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	cor.	$\delta^{18}O$ H_2O meas.	avg.
		0				+18.1	
7J		6	0.37	+3.4	+6.6	+19.3	+18.7
16J	0.4 ml	13	0.38	+5.7	+13.0	+19.9	+19.1
25J		27	0.45	+5.0	+8.9	+20.8	+19.5
34J	$H_2{}^{18}O$	41	0.51	+6.7	+11.3	+22.0	+20.0
43J		55	0.61	+7.5	+11.4	+22.1	+20.4
		0				+32.7	
8J		6	0.35	+4.6	+11.3	+33.1	+32.9
17J	0.6 ml	13	0.34	+7.0	+21.1	+34.4	+33.4
26J		27	0.43	+8.1	+16.8	+34.3	+33.6
35J	$H_2{}^{18}O$	41	0.52	+11.2	+20.0	+35.3	+34.0
44J		55	0.60	+12.8	+20.5	+36.4	+34.4
		0				+44.9	
9J		6	0.35	+4.8	+11.5	+46.0	+45.5
18J	0.8 ml	13	0.37	+6.7	+16.6	+47.1	+46.0
27J		27	0.41	+10.9	+25.0	+47.2	+46.3
36J	$H_2{}^{18}O$	41	0.51	+14.1	+26.0	+47.7	+46.6
45J		55	0.63	+18.7	+29.8	+49.4	+47.1
		0				+60.0	
10J		6	0.37	+7.5	+19.0	+60.0	+60.0
19J	1.0 ml	13	0.39	+9.3	+22.7	+60.6	+60.2
28J		27	0.43	+12.4	+26.8	+62.1	+60.7
37J	$H_2{}^{18}O$	41	0.52	+18.8	+34.2	+62.6	+61.1
46J		55	0.65	+21.5	+33.7	+63.4	+61.5

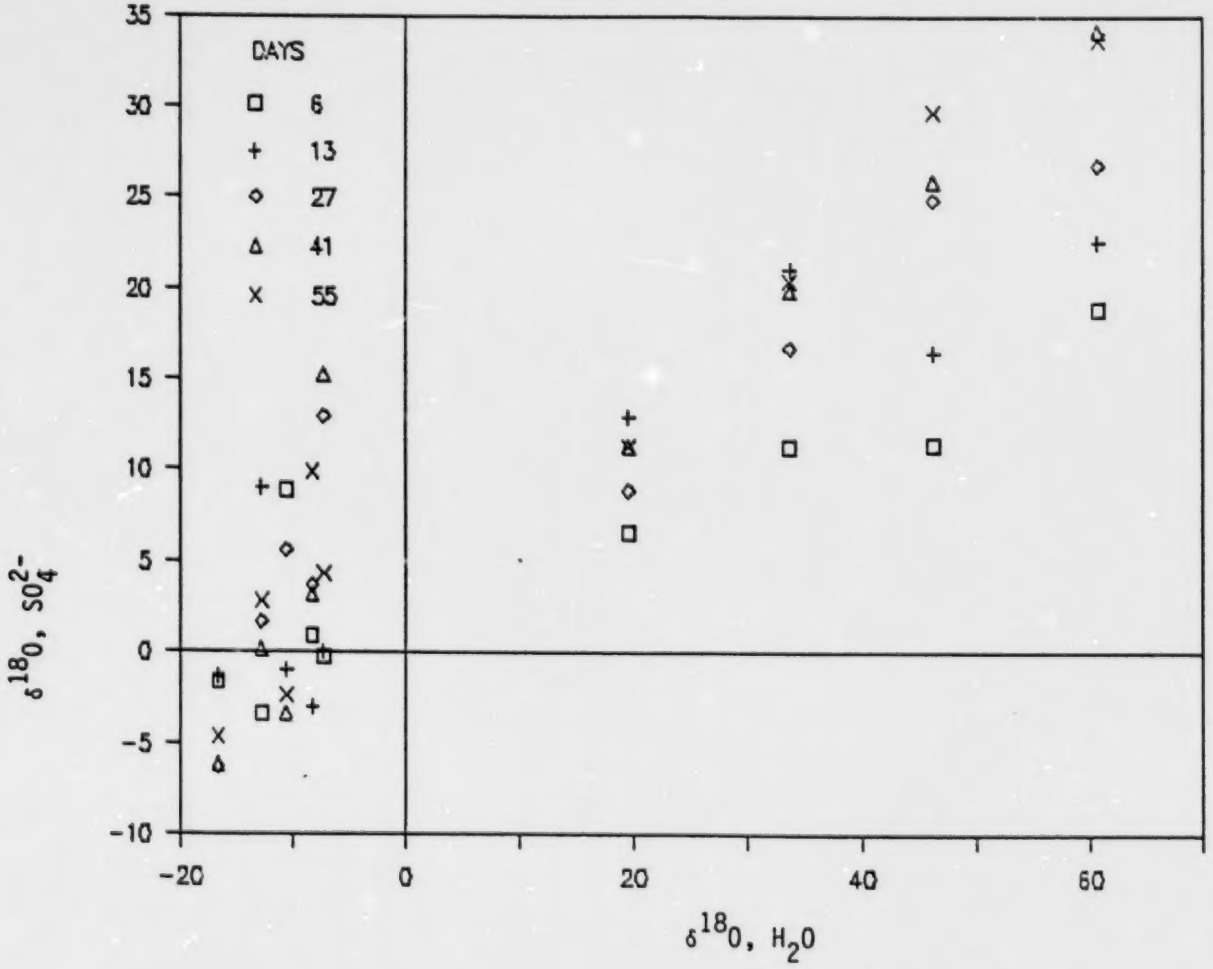

Figure 4 $\delta^{18}O(SO_4^{2-})$ versus $\delta^{18}(H_2O)$

Experiment #11 Oxidation of Chalcopyrite

TABLE 12 Experiment #12

OXIDATION OF PENTLANDITE PLUS CHALCOPYRITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	cor.	H_2O meas.	avg.
1K Inoculum		0	1.33	+2.1		-17.4	
		0				-17.4	
2K		7	0.79	-6.6	-8.4	-16.7	-17.1
11K	900 ml	14	1.01	-3.1	-3.9	-15.6	-16.6
20K	Calgary	21	1.13	-1.5	-2.0	-15.7	-16.4
29K	H_2O	28	1.20	-1.6	-2.1	-15.0	-16.1
38K		35	1.26	+4.2	+4.4	-13.4	-15.6
47K		42	1.32	-3.8	-4.5	-12.9	-15.2
		0				-15.0	
3K		7	0.76	-4.1	-5.4	-13.5	-14.3
12K	600 ml	14	0.97	+1.6	+1.5	-12.7	-13.7
21K	Calgary	21	1.16	+2.4	+2.4	-12.9	-13.5
30K	H_2O	28	1.18	-0.2	-0.5	-11.2	-13.1
39K		35	1.28	+2.8	+2.9	-10.8	-12.7
48K		42	1.30	-2.7	-3.2	-9.9	-12.3
		0				-11.9	
4K		7	0.78	-1.8	-2.6	-10.9	-11.4
13K	300 ml	14	0.94	+1.8	+1.8	-11.1	-11.3
22K	Calgary	21	0.49	+0.5	+0.3	-9.4	-10.8
31K	H_2O	28	1.21	+2.9	+3.0	-8.2	-10.3
40K		35	1.26	+2.9	+3.0	-8.5	-10.0
49K		42	1.34	-1.8	-2.2	-7.8	-9.7
		0				-9.1	
5K		7	0.79	+2.7	+2.8	-8.1	-8.6
14K	Ottawa	14	0.88	+4.4	+4.8	-7.8	-8.3
23K	Water	21	1.08	+0.7	+0.5	-7.9	-8.2
32K		28	1.17	+3.5	+3.7	-6.9	-8.0
41K		35	1.28	+0.3	+0.1	-5.6	-7.6
50K		42	1.36	+4.1	+4.3	-4.9	-7.2

TABLE 12 Experiment #12 CONTINUED

OXIDATION OF PENTLANDITE PLUS CHALCOPYRITE

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	cor.	$\delta^{18}O$ H_2O meas.	avg.
		0				+6.3	
6K		7	0.75	+9.8	+11.4	+4.3	+5.3
15K	0.2 ml	14	0.86	+10.5	+12.0	+4.6	+5.1
24K		21	0.80	+15.2	+17.8	+4.5	+4.9
33K	$H_2{}^{18}O$	28	1.10	+9.3	+10.3	+5.3	+5.0
42K		35	1.24	+8.4	+9.7	+5.8	+5.1
51K		42	1.24	+10.3	+11.3	+7.2	+5.4
		0				+15.1	
7K		7	0.73	+8.1	+21.7	+15.6	+15.4
16K	0.4 ml	14	0.90	+21.2	+24.5	+17.0	+15.9
25K		21	1.06	+18.3	+20.6	+17.4	+16.3
34K	$H_2{}^{18}O$	28	1.13	+17.3	+19.3	+17.7	+16.6
43K		35	1.28	+17.9	+19.7	+18.6	+16.9
52K		42	1.31	+19.2	+21.1	+19.9	+17.3
		0				+26.8	
8K		7	0.68	+23.8	+29.1	+27.9	+27.4
17K	0.6 ml	14	0.86	+26.8	+31.3	+28.2	+27.6
26K		21	1.01	+25.7	+29.3	+28.4	+27.8
35K	$H_2{}^{18}O$	28	1.10	+25.3	+28.5	+28.7	+28.0
44K		35	1.27	+26.6	+29.5	+29.9	+28.3
53K		42	1.37	+26.3	+28.9	+30.7	+28.7
		0					
9K		7	0.67	+31.8	+39.1	+40.2	+39.3
18K	0.8 ml	14	0.85	+32.5	+38.1	+40.3	+39.6
27K		21	1.01	+34.8	+39.8	+40.4	+39.8
36K	$H_2{}^{18}O$	28	1.12	+32.8	+37.0	+40.5	+40.0
45K		35	1.25	+37.2	+41.4	+43.3	+40.5
54K		42	1.36	+33.8	+37.2	+43.2	+40.9
		0				+54.4	
10K		7	0.68	+40.4	+49.6	+55.8	+55.1
19K	1.0 ml	14	0.94	+36.6	+42.3	+56.4	+55.5
28K		21	1.04	+39.3	+44.8	+56.8	+55.9
37K	$H_2{}^{18}O$	28	1.11	+41.6	+47.0	+57.0	+56.1
46K		35	1.26	+43.0	+47.8	+58.1	+56.4
55K		42	1.41	+45.7	+50.2	+57.8	+56.6

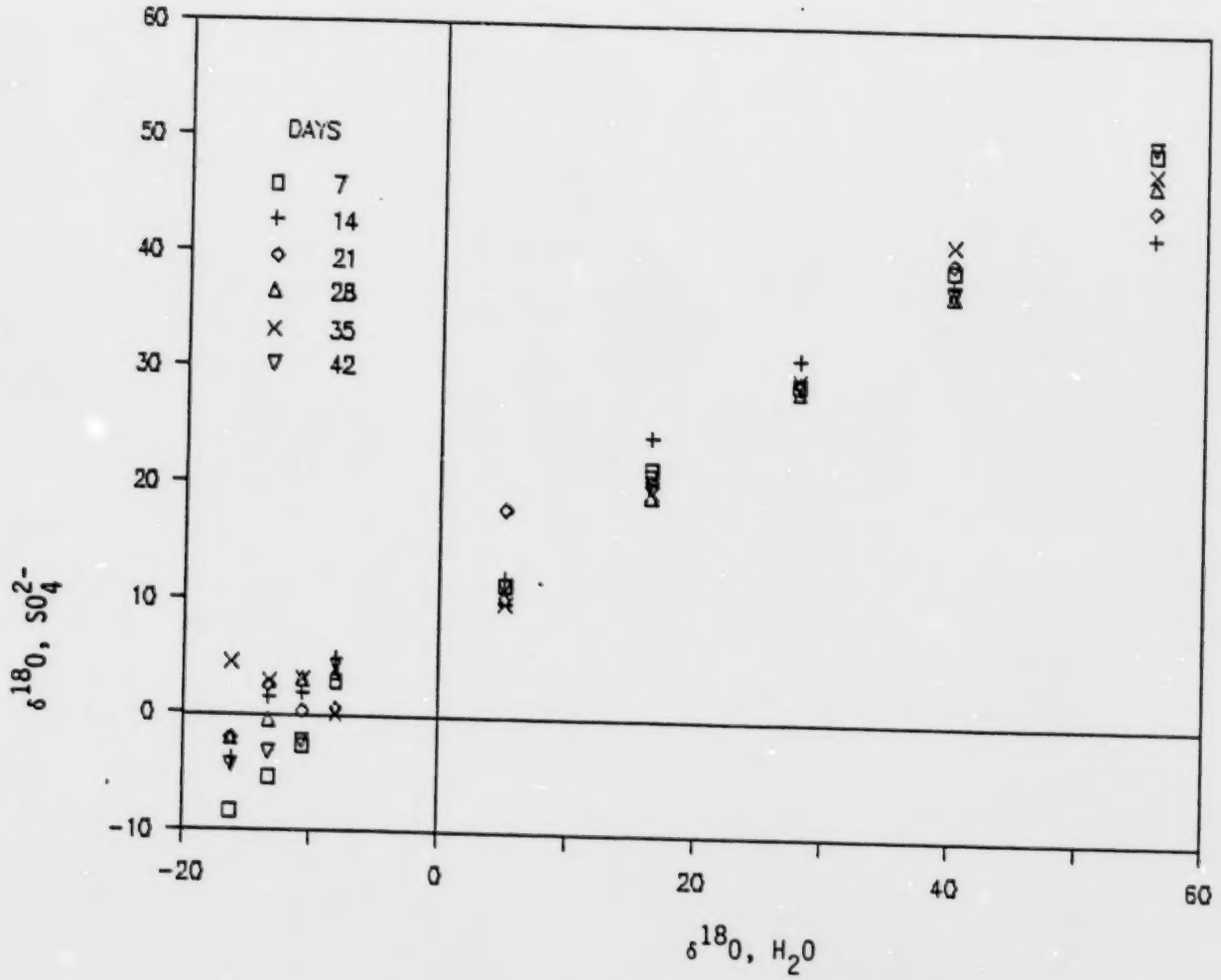

Figure 5 $\delta^{18}O(SO_4^{2-})$ versus $\delta^{18}(H_2O)$

Experiment #12 Oxidation of Pentlandite plus Chalcopyrite

TABLE 13 Experiment #13

OXIDATION OF PYRITE WITH ISOLATE SM-4

Sample		Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	$\delta^{18}O$ SO_4^{2-} cor.	$\delta^{18}O$ H_2O meas.	$\delta^{18}O$ H_2O avg.
1L Inoculum		0	2.65	+1.4		-17.7	
		0				-17.7	
2L		7	0.52	-2.7	-7.0	-16.6	-17.2
11L	900 ml	14	1.07	-6.0	-8.4	-15.6	-16.6
20L	Calgary	21	1.55	-5.8	-7.3	-15.5	-16.4
29L	H_2O	28	2.01	-6.3	-7.5	-14.2	-15.9
38L		35	2.19	-4.2	-5.0	-13.7	-15.6
		0				-15.1	
3L		7	0.53	-3.8	-9.0	-14.0	-14.6
12L	600 ml	14	1.06	-4.8	-6.9	-13.2	-14.1
21L	Calgary	21	1.42	-6.5	-8.3	-13.3	-13.9
30L	H_2O	28	2.01	-7.3	-8.6	-12.8	-13.7
39L		35	1.89	-3.4	-4.2	-11.5	-13.3
		0				-12.2	
4L		7	0.52	-1.9	-5.3	-11.0	-11.6
13L	300 ml	14	1.12	-4.6	-6.5	-11.0	-11.4
22L	Calgary	21	1.34	-4.7	-6.2	-10.2	-11.1
31L	H_2O	28	2.02	+0.6	+0.5	-8.9	-10.7
40L		35	1.76	-7.0	-8.5	-7.8	-10.2
		0				-10.1	
5L		7	0.51	-1.9	-5.5	-9.0	-9.6
14L	Ottawa	14	1.07	-0.7	-1.4	-8.4	-9.2
23L	Water	21	1.35	+3.4	+3.9	-7.6	-8.8
32L		28	1.79	-0.2	-0.5	-6.6	-8.3
41L		35	1.77	-5.2	-6.4	-5.9	-7.9
		0				+3.3	
6L		7	0.53	+2.1	+2.8	+3.8	+3.6
15L	0.2 ml	14	1.15	+6.8	+8.4	+4.6	+3.9
24L		21	1.45	+10.4	+12.4	+4.6	+4.1
33L	$H_2{}^{18}O$	28	1.84	+2.3	+2.5	+6.1	+4.5
42L		35	2.18	+3.7	+4.0	+6.8	+4.9

TABLE 13 Experiment #13 CONTINUED

OXIDATION OF PYRITE WITH ISOLATE SM-4

Sample	Time(Days)	SO_4^{2-} (mmol/mg)	$\delta^{18}O$ SO_4^{2-} meas.	$\delta^{18}O$ SO_4^{2-} cor.	$\delta^{18}O$ H_2O meas.	$\delta^{18}O$ H_2O avg.
	0				+18.2	
7L	7	0.53	+11.8	+22.1	+19.4	+18.8
16L 0.4 ml	14	1.09	+14.0	+18.0	+20.2	+19.3
25L	21	1.36	+14.7	+17.9	+20.4	+19.6
34L $H_2{}^{18}O$	28	1.77	+15.1	+17.5	+21.0	+19.8
43L	35	2.07	+14.4	+16.0	+21.5	+20.1
	0				+26.6	
8L	7	0.49	+12.7	+26.3	+27.8	+27.2
17L 0.6 ml	14	0.98	+21.8	+29.3	+28.5	+27.6
26L	21	1.40	+19.6	+23.9	+29.2	+28.0
35L $H_2{}^{18}O$	28	1.57	+18.2	+21.6	+29.8	+28.4
44L	35	2.11	+22.0	+25.0	+29.7	+28.6
	0				+41.0	
9L	7	0.52	+17.3	+34.1	+41.9	+41.5
18L 0.8 ml	14	1.05	+25.4	+33.5	+42.2	+41.7
27L	21	1.48	+26.6	+32.1	+42.7	+42.0
36L $H_2{}^{18}O$	28	1.88	+27.6	+31.9	+44.4	+42.4
45L	35	2.29	+33.1	+37.3	+44.3	+42.8
	0				+60.0	
10L	7	0.53	+27.4	+53.5	+60.6	+60.3
19L 1.0 ml	14	1.03	+36.7	+48.9	+61.8	+60.8
28L	21	1.36	+32.7	+40.3	+61.9	+61.1
37L $H_2{}^{18}O$	28	1.70	+37.6	+44.4	+61.7	+61.2
46L	35	2.18	+38.4	+43.5	+62.4	+61.4

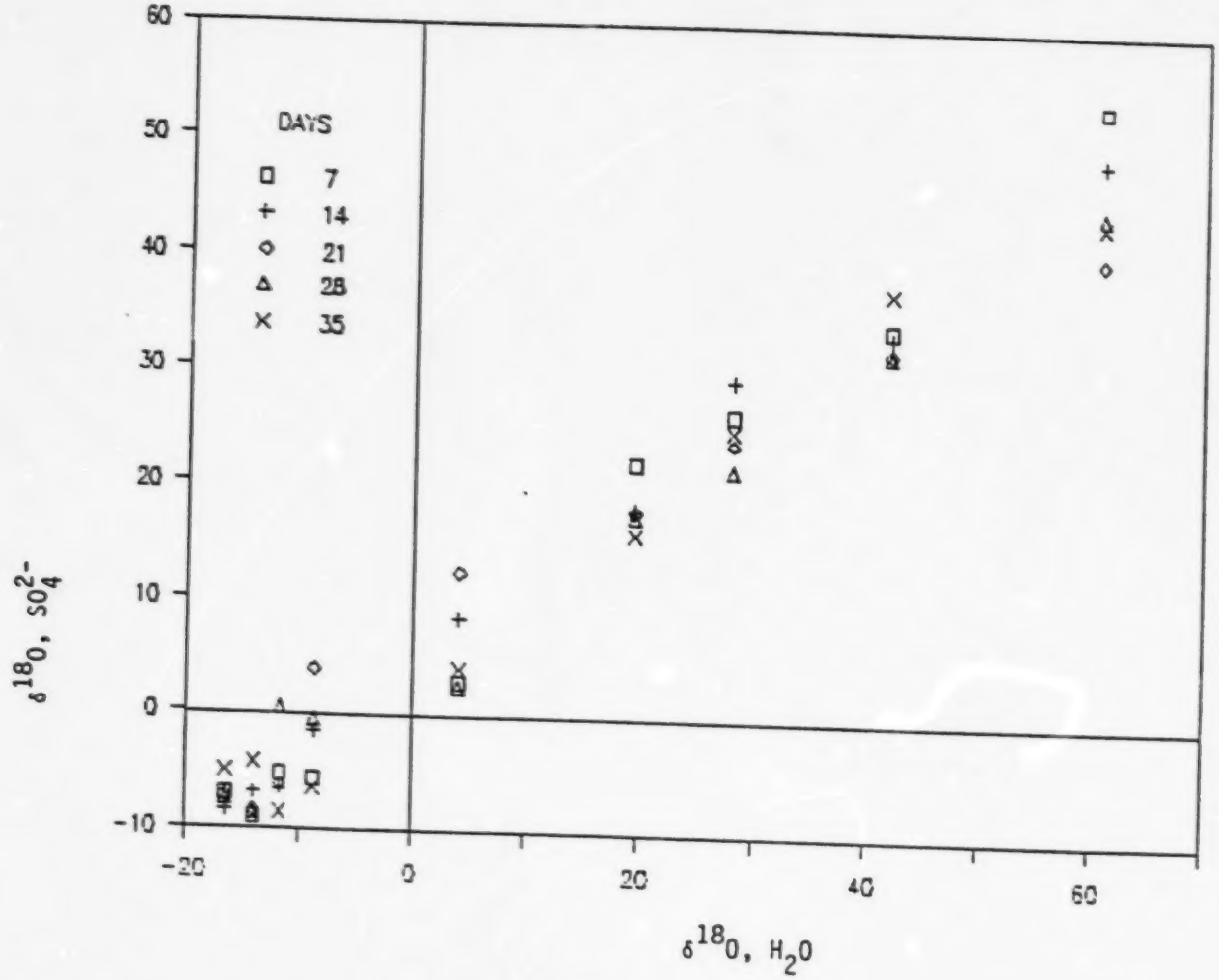

Figure 6 $\delta^{18}O(SO_4^{2-})$ versus $\delta^{18}(H_2O)$

Experiment #13 Oxidation of Pyrite with Isolate SM-4

TABLE 14

Dependence of Oxygen Isotope Composition of Sulphate on that of Water

Equation $\delta^{18}O\ (SO_4^{2-}) = m\ \delta^{18}O\ (H_2O) + b$

	Days	m	b	r^2
Experiment 8	6	0.55 ± 0.02	2.3 ± 1.6	0.987
Oxidation of	13	0.63 ± 0.02	4.5 ± 1.5	0.992
Pyrite	20	0.66 ± 0.03	4.5 ± 2.1	0.985
	27	0.73 ± 0.02	3.1 ± 1.5	0.994
	34	0.75 ± 0.02	3.6 ± 1.1	0.997
	Overall	0.66 ± 0.02	3.6 ± 2.6	0.973
Experiment 9	6	0.50 ± 0.02	5.9 ± 1.6	0.987
Oxidation of	13	0.64 ± 0.03	5.4 ± 2.2	0.985
Sphalerite	20	0.59 ± 0.03	8.2 ± 2.1	0.983
	27	0.61 ± 0.04	7.5 ± 2.9	0.971
	34	0.53 ± 0.05	9.8 ± 3.8	0.944
	Overall	0.57 ± 0.02	7.7 ± 3.2	0.949
Experiment 10	6	0.68 ± 0.01	7.1 ± 1.0	0.997
Oxidation of	13	0.70 ± 0.01	7.2 ± 0.9	0.998
Pentlandite	20	0.69 ± 0.03	7.0 ± 1.8	0.989
	27	0.75 ± 0.02	6.5 ± 1.6	0.993
	Overall	0.70 ± 0.01	7.0 ± 1.4	0.993
Experiment 11	6	0.23 ± 0.05	3.1 ± 4.1	0.740
Oxidation of	13	0.32 ± 0.07	4.5 ± 5.2	0.767
Chalcopyrite	27	0.38 ± 0.05	5.0 ± 3.9	0.896
	41	0.48 ± 0.05	4.6 ± 3.6	0.941
	55	0.46 ± 0.06	5.3 ± 4.6	0.901
	Overall	0.37 ± 0.03	4.5 ± 5.0	0.803
Experiment 12	7	0.80 ± 0.02	7.2 ± 1.7	0.974
Oxidation of	14	0.66 ± 0.04	10.0 ± 2.9	0.975
Pentlandite	21	0.68 ± 0.04	9.8 ± 3.0	0.975
plus	28	0.68 ± 0.01	8.8 ± 1.0	0.997
Chalcopyrite	35	0.68 ± 0.04	9.9 ± 3.4	0.968
	42	0.76 ± 0.02	7.1 ± 1.4	0.995
	Overall	0.71 ± 0.01	8.8 ± 2.6	0.978
Experiment 13	7	0.80 ± 0.03	3.7 ± 2.6	0.987
Oxidation of	14	0.75 ± 0.03	4.3 ± 2.2	0.991
Pyrite with	21	0.63 ± 0.05	4.7 ± 3.7	0.962
Isolate SM-4	28	0.67 ± 0.03	3.3 ± 2.6	0.983
	35	0.71 ± 0.04	2.5 ± 3.4	0.974
	Overall	0.71 ± 0.02	3.7 ± 3.2	0.971

DISCUSSION

In experiments where sulphur isotope compositions were determined, the sulphate was found to have $\delta^{34}S$ values close to that of the reactant sulphide. This implies that the rate controlling step was one with little or no sulphur isotope fractionation. One possibility is a surface reaction which proceeds layer by layer, and therefore provides negligible isotope selectivity.

In all experiments, the oxygen isotope composition of the sulphate "tracked" that of the water. It is noted that Experiments No. 1 and 7 were carried out with effectively the same water. Added labelled water did not have the designated ^{18}O enrichment. These experiments were nevertheless useful from the statistical viewpoint. Experiments No. 8 to 13 had $\delta^{18}O$ values for H_2O ranging up to $80^{o}/oo$.

In some experiments, there was considerably more variability than in others, the worst being Experiment No. 11. Experiment No. 11 with chalcopyrite also had the slowest rate of conversion and the lowest slope (~0.4) on the $\delta^{18}O$(sulphate) versus $\delta^{18}O(H_2O)$ plot. In some experiments, corrections for the sulphate carry over from the inoculum were required. Since this correction was the greatest for Experiment No. 11, mathematical tests were made to determine how an erroneous isotopic determination for the inoculum would affect the results. It is seen in Table 15 that an error of $4^{o}/oo$ in the $\delta^{18}O$ determination for the inoculum does not alter the slope but changes the intercept significantly. It is noted that the slope is more indicative of the extent to which oxygen from water is incorporated into the sulphate so error in measuring the inoculum's isotope composition does not detract from the interpretation.

TABLE 15 Experiment #11

Oxidation of Chalcopyrite Dependence of Oxygen Isotope Composition of Sulphate on that of water: Effect of error in $\delta^{18}O$ determination for inoculum

	Time (Days)	$\delta^{18}O$ Inoculum		
		Measured	Test Values	
		+1.9	0	-2.0
Slope	6	0.23 ± 0.05	0.23 ± 0.05	0.23 ± 0.05
Intercept		3.1 ± 4.1	7.3 ± 4.0	11.7 ± 3.9
R^2		0.740	0.753	0.764
Slope	13	0.32 ± 0.07	0.33 ± 0.07	0.34 ± 0.07
Intercept		4.5 ± 5.2	8.0 ± 5.2	11.7 ± 5.8
R^2		0.767	0.762	0.755
Slope	27	0.38 ± 0.05	0.39 ± 0.05	0.39 ± 0.05
Intercept		5.0 ± 3.9	7.4 ± 4.0	9.9 ± 4.0
R^2		0.896	0.897	0.898
Slope	41	0.48 ± 0.05	0.48 ± 0.05	0.48 ± 0.05
Intercept		4.6 ± 3.6	6.4 ± 3.6	8.3 ± 3.6
R^2		0.941	0.941	0.941
Slope	55	0.46 ± 0.06	0.46 ± 0.06	0.46 ± 0.06
Intercept		5.3 ± 4.6	6.62 ± 4.6	8.0 ± 4.5
R^2		0.901	0.900	0.900
Slope	Overall	0.37 ± 0.03	0.38 ± 0.03	0.38 ± 0.03
Intercept		4.5 ± 5.0	7.2 ± 4.7	10.0 ± 4.6
R^2		0.803	0.824	0.827

Weighing of precipitates introduces errors which accumulate as samples are sequentially removed. Since the sulphur isotope fractionation is small, the experiment could be designed so that the $\delta^{34}S$ value for the inoculum differed greatly from that of the metal sulphide. Then sulphur isotope measurements could be used to correct the $\delta^{18}O$ data for inoculum sulphate.

It is seen in Table 14 that the slope of the $\delta^{18}O$(sulphate) versus $\delta^{18}O(H_2O)$ line decreases in the order pentlandite ≈ pyrite, sphalerite, chalcopyrite. This qualitatively represents the order of decreasing derivation of sulphate oxygen from the water.

One important observation in Table 14 is the marked similarities in slopes and intercepts in Experiments No. 8 and No. 13. Both involved the oxidation of pyrite but two different bacterial isolates with quite different histories were used. This suggests that one need not be concerned that choice of isolate will influence the isotopic selectivity.

Another observation in Table 14 is the consistency of the isotopic data when a mixture of metal sulphides was oxidized. When a mixture of pentlandite and chalcopyrite was used (Experiment No. 12), both the slope and intercept were the same as in Experiment No. 10, using pentlandite alone. This is consistent with the fact that conversion of chalcopyrite in Experiment No. 11 was found to be very slow.

Although the slope is a measure of incorporation of H_2O oxygen into sulphate, the determination of this quantity is not straightforward. It is dependent upon the reaction mechanism and attending isotopic selectivity. In the case of pyrite, three oxidation reactions have been emphasized in the literature. These are summarized by van Everdingen and Krouse (1985) in the included reprint. With this approach, the

$\delta^{18}O$(sulphate) versus $\delta^{18}O(H_2O)$ line is used to assess the extent to which various assumed reactions are involved. If current data for pyrite oxidation are used in the diagram of van Everdingen and Krouse (1985), it is found that the reaction which involves only water is about 75 percent dominant (Fig. 7).

It must be stressed that the approach outlined above is very qualitative. There has been considerable discussion as to whether this simple approach based on stoichiometry is a realistic method of describing complex mechanisms. Similar reactions could be devised for the oxidation of other metal sulphides. These in turn may suggest why there are differences in slopes and intercepts for the $\delta^{18}O$(sulphate) versus $\delta^{18}O(H_2O)$ plots for the various minerals. However, if the goal is to fully understand the isotope systematics, reaction intermediates should be isolated and examined.

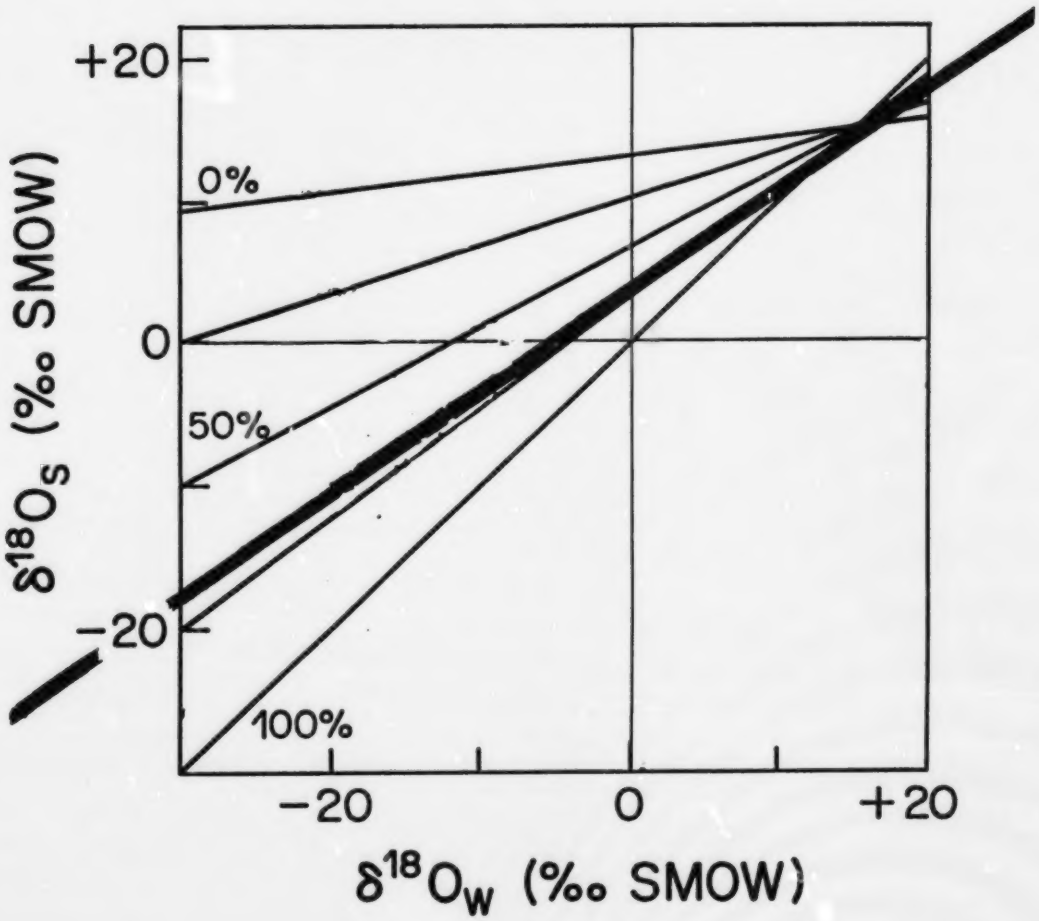

Figure 7. Comparison to data from current pyrite oxidation experiments with summary diagram of van Everdingen and Krouse (1985).

Reprinted from Nature, Vol. 315, No. 6018, pp. 395-396, 30 May 1985
© Macmillan Journals Ltd., 1985

Isotope composition of sulphates generated by bacterial and abiological oxidation

Robert O. van Everdingen

National Hydrology Research Institute, 4616 Valiant Drive, Calgary, Alberta, Canada T3A 0X9

H. Roy Krouse

Physics Department, The University of Calgary, Calgary, Alberta, Canada T2N 1N4

Oxygen isotope compositions of secondary sulphates provide a potentially powerful tool for elucidating mechanisms of sulphur oxidation. Such studies should reveal the consequences of various anthropogenic sulphur contributions to the environment, including gaseous emissions, aqueous solutions and elemental sulphur dust. At mining sites, metal sulphides may be oxidized *in situ*, giving rise to acid mine waters. The environment can receive these compounds from natural sources also, for example volcanoes, sulphurous springs and sour-gas seeps. There are many fundamental questions concerning the specific oxidation reactions and associated kinetics. In principle, isotope data from appropriate experiments can be used to address such questions. In the case of acid mine drainage, the approach of Taylor *et al.*[1] is noteworthy. Here we present a critical examination of their results and suggest a novel method of determining the kinetic isotope effects identified with the incorporation of O_2 and H_2O-oxygen by SO_4^{2-}.

Oxygen isotope data may provide information about redox reactions in the sulphur cycle when sulphur isotope data fail to do so. For example, SO_4^{2-} in subsurface water may be partially reduced to HS^- in deeper anaerobic enviroments, and the reduced product may be subsequently re-oxidized near the surface. The sulphur isotope composition may be conserved if processes such as metallic sulphide deposition or H_2S escape are absent; in this case there would be no sulphur-isotope evidence of the reduction-re-oxidation sequence. However, during re-oxidation, oxygen incorporated in the product SO_4^{2-} can be derived from both O_2 and H_2O molecules; this generally produces $\delta^{18}O$ values different from those of the original SO_4^{2-}. The above scenario has been documented for sulphates in several springs in western Canada[2-4].

Taylor *et al.*[1] presented a scheme whereby a plot (their Fig. 2) of $\delta^{18}O$ values of SO_4^{2-} versus those of associated H_2O can be used to evaluate the relative participation of two specific oxidation reactions. In our laboratory, $^{18}O/^{16}O$ analyses have been done on sulphate produced by oxidation of biogenic H_2S (near sulphurous springs)[2,3] and of metal sulphides (in acidic springs)[3]. It would seem that the approach of Taylor *et al.*[1] could be applied to these data. However, when this approach was attempted, it became apparent that their Fig. 2 required refinement.

Taylor *et al.*[1] cited three reactions identified with the oxidation of FeS_2:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (1)$$

$$Fe^{2+} + \tfrac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \tfrac{1}{2}H_2O \quad (2)$$

$$FeS_2 + \tfrac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (3)$$

In reaction (1) all sulphate oxygen (O_s) is derived from water molecules, whereas in reaction (3) 87.5% of the sulphate oxygen is derived from molecular oxygen and 12.5% from water molecules. Sulphates produced by the two reactions have different $\delta^{18}O_s$ values because of the generally large difference between the $\delta^{18}O$ values of O_2 and H_2O ($\delta^{18}O_a$ and $\delta^{18}O_w$, respectively).

Even if reactions (1) and (3) apply, there are two reasons why the isotope composition of oxygen in the sulphate does not necessarily reflect the stoichiometric proportions. First, a reactant atom need not end up in the product; it may instead enter a solvent molecule or another ion, depending on the reaction mechanisms and isotope exchange reactions. Second, reaction (2), the rate-limiting step for reaction (1), generates H_2O with a $\delta^{18}O$ value similar to that of O_2, which may affect the isotope balance of reaction (1). In the absence of data bearing on these possibilities, the stoichiometry of reactions (1) and (3) will be assumed to apply to the isotope composition, as done by Taylor *et al.*[1].

In their Fig. 2, Taylor *et al.*[1] plotted $\delta^{18}O$ values for SO_4^{2-} in acid mine waters ($\delta^{18}O_s$) against those for associated H_2O ($\delta^{18}O_w$). They drew a line representing zero difference between $\delta^{18}O_s$ and $\delta^{18}O_w$, labelled 'H_2O sole O_2 source', and a parallel line, offset by about +3.5‰ and labelled 100%, representing sulphates produced exclusively by reaction (1). The offset reflects the estimated kinetic isotope effect for the reaction. Three further lines, corresponding to 75, 50 and 25% contribution by reaction (1) were drawn parallel to the first two lines.

When we plotted $\delta^{18}O_s$ values for dissolved sulphates from acidic spring waters in western Canada, associated with lower $\delta^{18}O_w$ values (−23‰ standard mean ocean water, SMOW) than the data of Taylor *et al.*[1], an unrealistic contribution from reaction (3) was indicated. On further reflection, it was realized that the lines labelled 75, 50 and 25% should not be parallel. This can be readily appreciated by assuming a hypothetical reaction whereby all oxygen in the product sulphate is derived from O_2. In that case, the plot would be a horizontal line, as $\delta^{18}O_s$ would be independent of $\delta^{18}O_w$. Whereas the line for 100% contribution of sulphate oxygen by reaction (1) should be parallel to the line for 'H_2O sole oxygen source' and have a slope of unity (Fig. 1), decreasing contributions by reaction (1) would produce lines with progressively decreasing slopes.

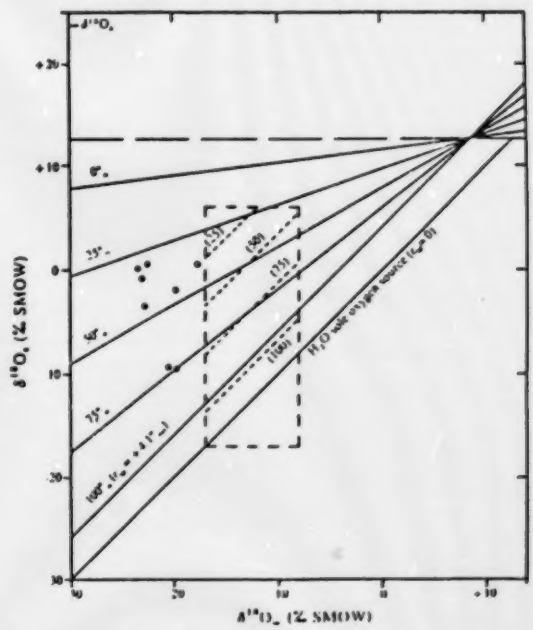

Fig. 1 Expected $\delta^{18}O_s$ values for secondary sulphates produced by oxidation of pyrite plotted against the $\delta^{18}O_w$ value of associated water, for relative contributions by reaction (1) ranging from 0 to 100%. Broken lines are from Taylor *et al.*[1]. Points represent data for acidic springs in western Canada.

The above considerations are incorporated in the isotope balance equation

$$\delta^{18}O_s = Y(\delta^{18}O_w + \varepsilon_w) + (1 - Y)$$
$$[0.875\,(\delta^{18}O_a + \varepsilon_a) + 0.125(\delta^{18}O_w + \varepsilon_w)] \quad (4)$$

in which Y is the fraction of SO_4^{2-} produced by reaction (1); ε_w and ε_a represent shifts in $\delta^{18}O_w$ and $\delta^{18}O_a$ values due to kinetic isotope effects during incorporation of O_w and O_a into sulphate. Using $\varepsilon_w = +4.1‰$ (ref. 1), $\varepsilon_a = -11.2‰$ (ref. 1) and $\delta^{18}O_a = +23.8‰$ (ref. 5), equation (4) can be rewritten as

$$\delta^{18}O_s = \delta^{18}O_w(0.875\,Y + 0.125) + (11.5375 - 7.4375\,Y) \quad (5)$$

Lines for 75, 50, 25 and 0% contribution by reaction (1), calculated using equation (5), are plotted in Fig. 1; they converge and intersect at the point where $\delta^{18}O_s = +12.6‰$ and $\delta^{18}O_w = +8.5‰$. This convergence means than at low latititudes, where $\delta^{18}O_w$ values are closer to SMOW, it is more difficult to differentiate contributions from individual reactions than at higher latitudes, where $\delta^{18}O_w$ values may be lower than $-30‰$.

The 75, 50 and 25% lines from Fig. 2 of Taylor *et al.*[1] have been added to our Fig. 1, together with $\delta^{18}O$ data from acidic springs in western Canada. It is readily seen that extrapolation of the parallel lines of Taylor *et al.*[1] would indicate very different proportions for the relative contributions by reactions (1) and (3) to these sulphates.

Figure 1 also suggests that oxidation of FeS_2 in contact with water having a $\delta^{18}O_w$ value of +8.5‰ should produce sulphate having a $\delta^{18}O_s$ value equal to +12.6‰, independent of the relative contributions by reactions (1) and (3). It should be possible to verify this point experimentally; this in turn would provide a check on the values for ε_a and ε_w, as well as on the validity of the assumption that the stoichiometry of reaction (3) applies, or whether the overall approach is too simplified.

Received 29 November 1984; accepted 28 March 1985.

1. Taylor, B. E., Wheeler, M. C. & Nordstrom, D. K. *Nature* **308**, 538–541 (1984).
2. van Everdingen, R. O., Shakur, M. A. & Krouse, H. R. *Can. J. Earth Sci.* **19**, 1395–1407 (1982).
3. Shakur, M. A. thesis, Univ. Calgary, Alberta (1982).
4. van Everdingen, R. O., Shakur, M. A. & Krouse, H. R. *4th Int. Symp. Water–Rock Interaction*, 526–529 (Okayama University Press 1983).
5. Honbe, Y., Shigehara, K. & Takakua, Y. *J. geophys. Res.* **78**, 2625–2629 (1973).

Printed in Great Britain by Macmillan Production Ltd, Basingstoke, Hampshire

II EXCHANGE OF OXYGEN ISOTOPES BETWEEN ARSENATE AND WATER

INTRODUCTION

Some sulphide oxidizing bacteria are capable of oxidizing arsenopyrite. It is important to better understand the fate of arsenic during this conversion. Considerations include (1) To what extent does the arsenic inhibit the sulphide oxidation? (2) Can the arsenopyrite conversion be optimized for more efficient recovery of precious metals? (3) What are the environmental consequences of this conversion?

Since arsenate is the most likely product of the oxidation, it was thought that the oxygen isotope composition of this ion might provide a means of studying the conversion, analagous to the use of oxygen isotopes in sulphate for studying sulphide oxidation. Such an approach is only feasible if the rate of exchange of oxygen isotopes between arsenate and water is much slower than the rates of bacterial oxidation. Therefore experiments were designed to measure the oxygen isotope exchange rates.

PROCEDURES

A 100 mM solution of $Na_2HAsO_4 \cdot 7H_2O$ was made up and divided into 60 ml aliquots. A different amount of water highly enriched in ^{18}O was added to each aliquot. The initial pH of the solutions was 8.74. After various times, 5 ml of each solution was taken to measure the oxygen isotope composition of the water. Another 5 ml was taken and pipetted into 50 ml of 1% $AgNO_3$. This precipitated Ag_3AsO_4 which was filtered and dried. This compound was chosen because it is one of few arsenate compounds with no water of crystallization which would interfere with the oxygen isotope determination.

Oxygen in the arsenate was converted to CO_2 by reaction with guanidine. Guanidine hydrochloride has been used for many years to convert water to CO_2 in oxygen isotope labelling experiments (cf Xu Fan-Chou et al. 1985). In our laboratory, there has been an ongoing programme to use this reagent for converting a variety of oxygen compounds to CO_2 (Manuscript in preparation).

RESULTS

Data for samples taken after one half hour and seven days are given in Table 16 and Fig. 18. It is clear that the exchange proceeds very rapidly and is substantial at the end of one half hour, the time typically taken for dissolution of $Na_2HAsO_4 \cdot 7H_2O$ followed by immediate precipitation of Ag_3AsO_4.

It is noted that the 50 ml solutions of $AgNO_3$ did not possess the ^{18}O enrichment of the arsenate solutions. Exchange during the short time for precipitation of Ag_3AsO_4 may have lowered the $\delta^{18}O$ values slightly thereby underestimating the extent of exchange.

CONCLUSION

The rate of oxygen exchange between arsenate and water was found to be very rapid with a half time of the order of 1 week. The pH dependence of this exchange has not been addressed in this preliminary study. However, in typical experiments, it seems highly unlikely that the oxygen isotope composition of arsenate could be used to elucidate bacterial oxidation mechanisms.

Table 16 Oxygen Isotope Composition of Components during an Arsenate-Water Exchange Experiment

		$\delta^{18}O$	
SAMPLE	H_2O	Ag_3AsO_4 Precipitate	
		1 hr	7 days
AS-1	-9.9	+11.2	+4.5
AS-2	+127	+14.8	+43.6
AS-3	+223	+25.0	+66
AS-4	+454	+36.1	+140
AS-5	+685	+47.4	+218
AS-6	+906	+69.2	+273
AS-7	+1109	+86.9	+341
INITIAL $NaHAsO_4 \cdot 7H_2O$		+6.7	

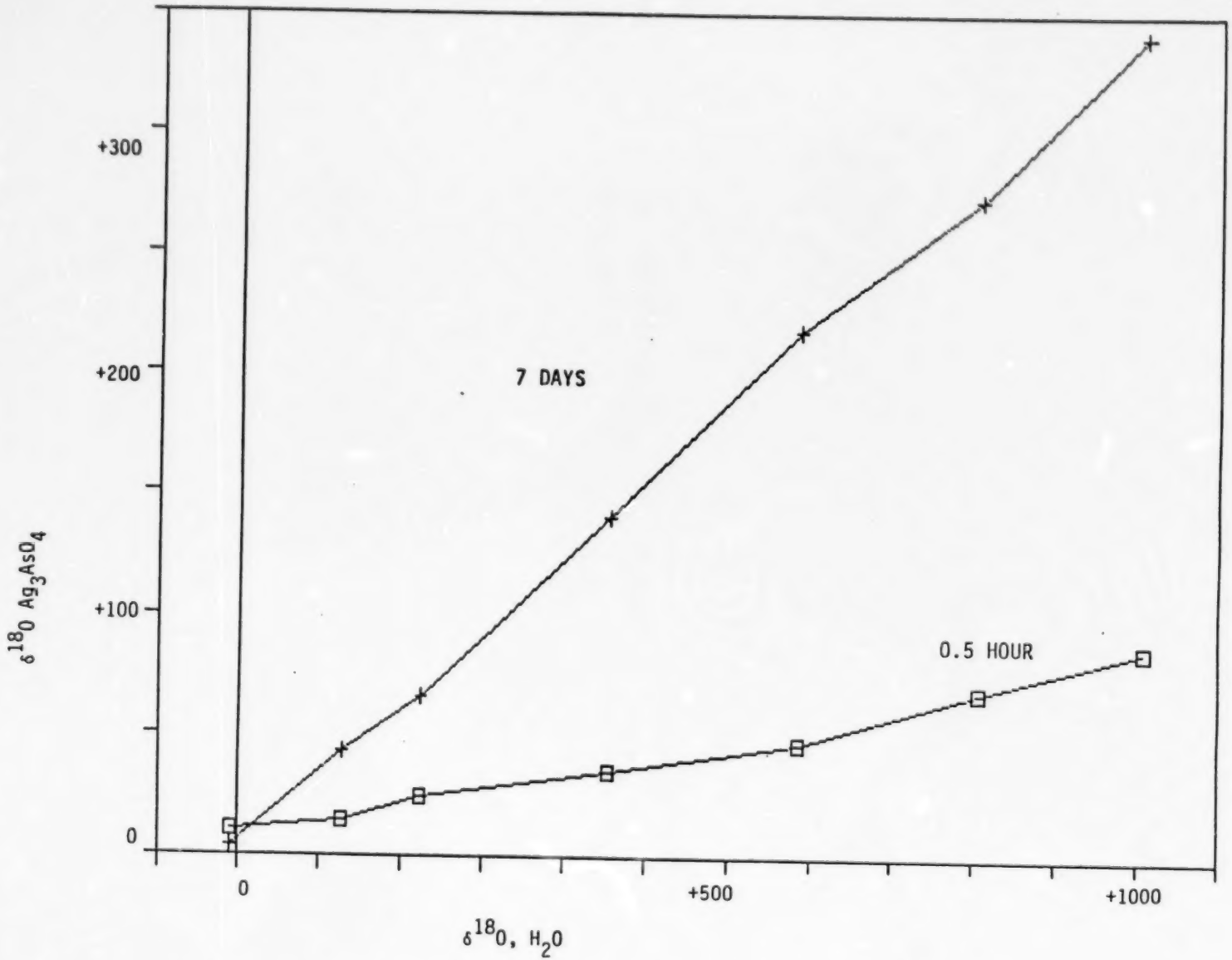

Figure 8 Oxygen isotope exchange between arsenate and water

REFERENCES

1. Lloyd, R.M. 1968. Oxygen isotope behavior in the sulfate - water system. J. Geophys. Res. 73: 6099-6110.

2. Sakai, H. and Krouse, H.R. 1971. Elimination of memory effects in $^{18}O/^{16}O$ determinations in sulphates. Earth and Planetary Science Letters 11: 369-373.

3. Taylor, B.E. and Wheeler, M.C. 1984a. Isotope composition of sulphate in acid mine drainage as measure of bacterial oxidation. Nature 308: 538-541.

4. Taylor, B.E., Wheeler, M.C., and Nordstrom, D.K. 1984b. Stable isotope geochemistry of acid mine drainage: experimental oxidation of pyrite. Geochim. Cosmochim. Acta 48: 2669-2678.

5. Ueda, A. and Krouse, H.A. 1987. Direct conversion of sulphide and sulphate minerals to SO_2 for isotope analyses. Geochemical Journal 20, 209-212.

6. Van Everdingen, R.O. and Krouse, H.R. 1985. Isotope composition of sulphates generated by bacterial and abiological oxidation. Nature 315: 395-396.

7. Xu Fan-Chou, Krouse, H.R., and Swaddle, T.W. 1985. Conjugate base pathway for water exchange on aqueous chromium III: Variable pressure and temperature kinetic study". Inorganic Chemistry 24: 267-270.